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Pharmacokinetics and Pharmacodynamics of Nandrolone Esters in Oil Vehicle: Effects of Ester, Injection Site and Injection Volume[1]

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ABSTRACT

We studied healthy men who underwent blood sampling for plasma nandrolone, testosterone and inhibin measurements before and for 32 days after a single i.m. injection of 100 mg of nandrolone ester in arachis oil. Twenty-three men were randomized into groups receiving nandrolone phenylpropionate (group 1, $n = 7$) or nandrolone decanoate (group 2, $n = 6$) injected into the gluteal muscle in 4 ml of arachis oil vehicle or nandrolone decanoate in 1 ml of arachis oil vehicle injected into either the gluteal (group 3, $n = 5$) or deltoid (group 4, $n = 5$) muscles. Plasma nandrolone, testosterone and inhibin concentrations were analyzed by a mixed-effects indirect response model. Plasma nandrolone concentrations were influenced ($P < .001$) by different esters and injection sites, with higher and earlier peaks with the phenylpropionate ester, compared with the decanoate ester. After nandrolone decanoate injection, the highest bioavailability and peak nandrolone levels were observed with the 1-ml gluteal injection. Plasma testosterone concentrations were also influenced ($P < .001$) by the ester and injection site, with the most rapid, but briefest, suppression being due to the phenylpropionate ester, whereas the most sustained suppression was achieved with the 1-ml gluteal injection. Plasma inhibin concentrations were also significantly influenced by injection volume and site, with the lowest nadir occurring after the nandrolone decanoate 1-ml gluteal injection. Thus, the bioavailability and physiological effects of a nandrolone ester in an oil vehicle are greatest when the ester is injected in a small (1 ml *vs.* 4 ml) volume and into the gluteal *vs.* deltoid muscle. We conclude that the side-chain ester and the injection site and volume influence the pharmacokinetics and pharmacodynamics of nandrolone esters in an oil vehicle in men.

For decades, administration of androgens such as testosterone and 19-nor-testosterone has been most frequently *via* depot i.m. injections of steroid esters dissolved in a vegetable oil vehicle (Junkman, 1957; Behre *et al.*, 1990). Such i.m. injections provide sustained androgen release into the circulation and have remained the mainstay of androgen replacement therapy for the last few decades (Nieschlag and Behre, 1990), although the basic pharmacological mechanisms are complex and only partially understood (Zuidema *et al.*, 1988). The basic pharmacology of this depot androgen formulation differs among species (van der Vies, 1965) but has been little studied in humans. The current understanding is that the rate-limiting mechanism governing the appearance of active steroid in the bloodstream is the retention of steroid esters from the oil vehicle depot due to oil/water partitioning, with gradual release into the extracellular fluid, where esters are rapidly hydrolyzed to liberate biologically active steroid. Other physiological and physico-chemical factors that could influence steroid appearance in the bloodstream include the chemistry of the side-chain ester (hydrophobicity, steric hindrance of hydrolysis and solubility), injection factors (depth, site and volume, pH and osmolarity of the solution), exercise and systemic illness. The influence of site and volume of injection on the release kinetics of androgen esters from oil vehicle depots has, however, not been systematically investigated in humans.

This study compared the pharmacokinetics and pharmacodynamics of two currently available esters of nandrolone, the decanoate and phenylpropionate, as well as the influence of i.m. injection sites (gluteal *vs.* deltoid) and injection volumes (4 ml *vs.* 1 ml). In addition to measuring plasma nandrolone to investigate pharmacokinetics, we measured plasma testosterone and inhibin by radioimmunoassay to determine the pharmacodynamic effects of nandrolone-induced inhibition of pituitary gonadotrophin secretion, as reflected in LH-dependent Leydig (testosterone) and FSH-dependent Sertoli (inhibin) cell function in healthy men. We analyzed these data

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ABBREVIATIONS: FSH, follicle-stimulating hormone; GAM, generalized additive model; LH, luteinizing hormone.

using an indirect pharmacodynamic response model, which has demonstrated, for the first time, prominent pharmacological differences between esters differing in only a single carbon in the side-chain, as well as systematic differences attributable to injection site and volume in humans.

Materials and Methods

Experimental design. Twenty-three healthy volunteers were randomly allocated into four groups in two balanced blocks. The first stratum of the study involved comparing two different nandrolone esters while controlling for dose, injection site and volume. In this stratum, volunteers received either nandrolone phenylpropionate (Durabolin; Organon) (group 1) or nandrolone decanoate (Deca-Durabolin; Organon) (group 2), administered as a single deep i.m. injection of 100 mg nandrolone ester into a single injection site (gluteal) in a fixed volume (4 ml of arachis oil vehicle). In the second stratum, a single ester (nandrolone decanoate) with fixed dose (100 mg) and volume (1 ml of arachis oil vehicle) was injected into two different sites, gluteal (group 3) or deltoid (group 4) muscles. This design also allowed comparison between different injection volumes (4 ml *vs.* 1 ml) for a single nandrolone ester with fixed dose and injection site (*i.e.,* group 2 *vs.* group 3).

Subjects. Healthy nonobese men, 18 to 40 years of age, with normal reproductive function, not competing in sports requiring International Olympic Committee-sanctioned urinary drug screening for anabolic steroids, not allergic to peanuts, free from chronic medical illness, not requiring regular prescribed medication and without abnormalities in routine clinical examination and biochemical screening were recruited. Fully informed written consent was obtained from volunteers, and the study had approval from the University of Sydney Human Ethics Committee within National Health and Medical Research Council Guidelines on Human Experimentation, which conform to the Declaration of Helsinki.

Procedures. Blood (5 ml) was sampled before injection (8:00–9:00 A.M.) and at 1, 2, 4, 6, 8 and 10 hr and 1, 2, 3, 4, 7, 9, 11, 14, 16, 18, 21, 23, 25, 28, 30 and 32 days after injection. During the first 10 hr, the participants were in a metabolic ward facility and were allowed to undertake normal daily activities. Subsequently, participants returned for blood sampling on an ambulatory basis while resuming their habitual daily activities.

To calculate absolute bioavailability, two additional subjects received a single i.v. bolus of 1 mg of 19-nor-testosterone, with sampling before injection and at 2, 4, 6, 8, 10, 15, 20, 30, 45, 60, 120, 180, 240, 360, 480, 720, 900, 1440, 1980 and 2880 min. For this study, crystalline nandrolone (Steraloids Inc., Wilton, NH) was dissolved in dehydrated ethanol BP (David Bull Laboratories, P/L, Mulgrave, Australia) at a concentration of 40 g/liter, from which a nandrolone stock solution (2 mg/ml in 15% ethanol) was produced by appropriate dilution with sterile saline. The nandrolone stock was filtered through a 0.2-μm cellulose acetate filter (Minisart NML; Sartorius GmBH, Gottingen, Germany), and the first 1 ml was discarded. Preliminary experiments showed that >80% of tracer nandrolone was recovered after such filtration. At the start of the study, the stock solution was diluted 10-fold with sterile normal saline in a 20-ml syringe (final ethanol concentration, 1.5%) and 5.0 ml was infused rapidly (within 10 sec) *via* an indwelling cannula in a forearm vein (nominal dose, 1.0 mg), followed by a flush of the syringe and cannula with an additional 5 ml of sterile saline solution. Aliquots of the injection solution were kept for subsequent assay of the exact amount of nandrolone administered. Blood sampling was from another cannula placed in the other arm.

Assays. Plasma samples were stored at −20°C until assay in a single batch per analyte. Steroids were measured by radioimmunoassay after extraction from plasma by a modification of a solid-phase method (Vining, 1980). Serum (200 μl for the nandrolone assay and 75 μl for the testosterone assay) was applied to a 5-cm mini-column of Extrelut (kieselguhr; Merck, Kilsyth, Australia) packed in a Pasteur pipette. Steroids were eluted by four washes of 750 μl of hexane/ethyl acetate (3:2) at 5-min intervals, the combined eluate was dried and the extract was reconstituted in assay buffer. Extraction efficiency was 97 ± 5% (n = 29) for testosterone and 90 ± 5% (n = 25) for nandrolone; therefore, no corrections were made for extraction losses. Radioactivity was measured with a Wallac 1410 liquid scintillation counter (Wallac Oy, Turku, Finland) and a LKB 1261 Multigamma gamma counter (Wallac Oy), using RIACALC data reduction software on an IBM-compatible computer. All steroid assay reagents were of analytical or higher grade.

Plasma nandrolone was measured by radioimmunoassay using a rabbit antibody to 19-nor-testosterone 17-hemisuccinate-bovine serum albumin (CER, Marloie, Belgium), tritiated tracer (19-[19-^{3}H]nor-testosterone; specific activity, 1.37 TBq/mmol; Amersham, North Ryde, Australia), nandrolone standard (Steraloids), extracts of plasma equivalent to 100 μl/tube and a dextran-charcoal separation of bound and free steroid. Cross-reactivities (expressed as molar ratios at the ED_{50}) with testosterone (0.04%), dihydrotestosterone (0.7%), androstenedione (0.3%), estradiol (0.02%), nandrolone phenylpropionate (6.3%) and nandrolone decanoate (1.3%) were negligible in relation to circulating levels. The assay detection limit (B/B_0 = 0.90) was 8 pg/tube (equivalent to 0.25 nM), and the ED_{50} was 100 pg/tube (equivalent to 3.5 nM). Coefficients of variation at low ($\approx ED_{80}$), medium ($\approx ED_{50}$) and high ($\approx ED_{20}$) levels of the standard curve (n = 16–24 assays) were 11.7, 9.8 and 11.9% (between-assay) and 6.2, 3.5 and 5.3% (within-assay), respectively.

Plasma testosterone was measured by radioimmunoassay using a rabbit antibody to testosterone-3-*O*-carboxymethyloxime-bovine serum albumin (SGT-1, supplied by Dr. B. Caldwell, Yale University), [1,2,6,7,16,17(N)-^{3}H]testosterone (specific activity, 5.00–6.66 TBq/mmol; DuPont, North Ryde, Australia), testosterone standard (Steraloids), extracts of plasma equivalent to 12.5 μl/tube and a dextran-charcoal separation of bound and free steroid. Correction was made for cross-reactivity with nandrolone (20.5%), but cross-reactivities with dihydrotestosterone (21.1%), androstenedione (2.5%), estradiol (0.17%), nandrolone phenylpropionate (0.04%) and nandrolone decanoate (<0.0002%) were negligible in relation to circulating levels. The assay detection limit (B/B_0 = 0.90) was 2 pg/tube (equivalent to 0.6 nM), and the ED_{50} was 22 pg/tube (equivalent to 6 nM). Coefficients of variation at low ($\approx ED_{80}$), medium ($\approx ED_{50}$) and high ($\approx ED_{20}$) levels on the standard curve (n = 8–32 assays) were 12.6, 17.1 and 12.9% (between-assay) and 8.3, 3.7 and 6.0% (within-assay), respectively.

Plasma inhibin was measured by a heterologous double-antibody radioimmunoassay established in our laboratory (Handelsman *et al.*, 1990; Crawford and Handelsman, 1994) and validated for humans (McLachlan *et al.*, 1990; Burger, 1992; Dong *et al.*, 1992; Wallace *et al.*, 1993). Reagents, provided by Dr. G. Bialy (Contraceptive Development Branch, National Institute of Child Health and Human Development), were rabbit antiserum to bovine inhibin (rAs-1989), purified 31-kDa inhibin from bovine follicular fluid for iodination (bINH-R-90/1) and bovine inhibin standard (bINH-R-90/1). Duplicate plasma samples (100 μl) were preincubated overnight with antibody before addition of ^{125}I-labeled inhibin. The standard curve was blanked with castrate human serum to equalize serum protein concentrations in the assay tubes. The detection limit (B/B_0 = 0.90) was 19.5 pg/ml, and coefficients of variation at inhibin standard levels of 90, 200 and 390 pg/ml were 3.1, 7.3 and 7.4% (between-assay) and 1.8, 3.0 and 5.0% (within-assay), respectively.

Data analysis. Plasma nandrolone, testosterone and inhibin levels were initially analyzed to evaluate the full time course for each hormone by repeated-measures analysis of variance with BMDP 5V software (Dixon, 1992), testing main effects by the Wald χ^2 test and using suitable linear contrasts to define effects of ester, injection site and volume. Due to the very different time courses of the phenylpropionate and decanoate esters, the three groups (groups 2–4) receiving the decanoate ester were also analyzed separately.

Plasma nandrolone levels were also analyzed by standard pharmacokinetic methods involving polyexponential curve fitting (Gibaldi and Perrier, 1982), with a weighted, nonlinear, least-squares, curve-fitting algorithm, by BMDP 3R software (Dixon, 1992). Standard pharmacokinetic parameters (time of peak, peak concentration, mean residence time, apparent half-times for absorption and clearance, systemic clearance and area under the curve) were derived empirically from the plasma nandrolone concentrations and as mathematical functions of the coefficients of the best-fit curve (Gibaldi and Perrier, 1982). Estimates of absolute bioavailability of nandrolone were calculated from the disappearance curves of plasma nandrolone after i.v. administration, by fitting to a variance-weighted, triexponential curve with corrections for the molecular weight of nandrolone (274.4) and its decanoate (428.7) and phenylpropionate (406.6) esters.

This pharmacokinetic analysis was subsequently extended by building a population pharmacokinetic model using the approach of Mandema *et al.* (1992). The plasma concentration of nandrolone was considered to be the convolution of a monoexponential or biexponential absorption function with a biexponential unit disposition function

$$\mathrm{UDF} = \sum_{i=1}^{2} A_i \cdot e^{-\lambda_i \cdot t} \tag{1}$$

The input rate (IR) for the monoexponential absorption model is given by

$$\mathrm{IR} = D \cdot F \cdot k \cdot e^{-k \cdot t} \tag{2}$$

where D is the dose, F is the fraction absorbed (bioavailability) and k is the first-order absorption rate constant. The input rate for the biexponential absorption model is given by

$$\mathrm{IR} = D \cdot F \cdot [P \cdot k_1 \cdot e^{-k_1 \cdot t} + (1 - P) \cdot k_2 \cdot e^{-k_2 \cdot t}] \tag{3}$$

where k_1 and k_2 are first-order absorption rate constants, P is the proportion of the bioavailable dose absorbed by k_1 and $(1 - P)$ is the proportion of the bioavailable dose absorbed by k_2. The convolution of these input functions with a biexponential disposition function yielded equations 4 and 5, which describe the plasma concentrations over time after i.m. injection for the monoexponential and biexponential absorption models, respectively.

$$Cp(t) = D \cdot F \cdot k \cdot \sum_{i=1}^{2} \frac{A_i}{k - \lambda_i} (e^{-\lambda_i \cdot t} - e^{-k \cdot t}) \tag{4}$$

$$Cp(t) = D \cdot F \cdot \sum_{i=1}^{2} \left[\frac{k_1 \cdot P \cdot A_i}{k_1 - \lambda_i} (e^{-\lambda_i \cdot t} - e^{-k_1 \cdot t}) + \frac{k_1 \cdot (1 - P) \cdot A_i}{k_2 - \lambda_i} (e^{-\lambda_i \cdot t} - e^{-k_2 \cdot t}) \right] \tag{5}$$

The population pharmacokinetic model did not include the data from the two subjects who received nandrolone i.v. Thus, parameter F was not identifiable and was incorporated into the models as $F{\cdot}A_1$ and $F{\cdot}A_2$. The interindividual error on each of the model parameters was modeled using a logarithmic-normal variance model

$$P_i = \theta_{\mathrm{TV}} e^{\eta_i}$$

where P_i is the value of the parameter in the individual, θ_{TV} is the typical value of the parameter in the population and η is a random variable with mean 0 and variance ω^2. The estimates of ω obtained with NONMEM are similar to the coefficient of variation for the parameter, which we report as the population variability, expressed as a percentage. We used a "constant coefficient of variation" model for the variance of the intraindividual residual error. Empirical Bayesian estimates of the individual pharmacokinetic parameters were obtained based on the typical values of the structural model parameters and on the variances of the interindividual errors. These population pharmacokinetic models were implemented with the computer program NONMEM (Beal and Sheiner, 1992).

We used a GAM (Chambers and Hastie, 1993) to permit identification of linear and nonlinear relationships between the Bayesian estimates of individual model parameters and the volunteer covariates (ester, site and volume of injection, group, age, weight, height, body surface area and lean body mass). The model that resulted in the biggest decrease in the Akaike information criterion was returned by the GAM function as the best model. The structural model incorporating covariates identified by the GAM analysis was then evaluated with NONMEM to develop the final parameter estimates. The final model was examined for parsimony by exclusion of individual covariates and demonstration of a statistically significant increase in the NONMEM objective function and by analysis of the standard errors of the estimated parameters.

Pharmacodynamics. The pharmacodynamic effects of nandrolone on plasma testosterone and inhibin were estimated by nadir concentration, time of nadir, net secretion and duration of suppression. The duration of suppression was defined as the time when plasma testosterone levels were below normal (<10 nM) or inhibin levels were reduced by 50% of base line. The effects of ester, injection site and volume were determined by parametric (testosterone) and nonparametric (inhibin) analysis of variance.

This pharmacodynamic analysis was extended by building a population pharmacodynamic model, again using the approach of Mandema *et al.* (1992). The pharmacodynamic model was based on one of the four basic indirect response models recently proposed by Dayneka *et al.* (1993). Because testosterone and inhibin secretion are both suppressed by nandrolone, model I of Dayneka *et al.* was physiologically the most appropriate to describe the pharmacodynamic effects of nandrolone and is shown in equation 6.

$$\frac{dR}{dt} = k_{\mathrm{in}}^0 \cdot \left[1 - \frac{Cp(t)}{Cp(t) + \mathrm{IC}_{50}} \right] - k_{\mathrm{out}} \cdot R(t) \tag{6}$$

R represents the measured response variable (either testosterone or inhibin concentrations), k_{in}^0 is a zero-order rate constant (the base-line testosterone or inhibin daily input rate), $Cp(t)$ is the plasma concentration of the inhibiting drug (nandrolone) as a function of time and IC_{50} is the drug concentration that results in 50% of maximum inhibition of the production rate. Under steady-state base-line conditions, it is noted that $k_{\mathrm{in}}^0 = k_{\mathrm{out}} \cdot R_0$, where R_0 is $R(t)$ at $t = 0$, reducing the number of parameters in the model. We have modified equation 6 to allow for incomplete inhibition of testosterone and inhibin synthesis by nandrolone and have included a parameter (γ) to describe the steepness of this relationship

$$\frac{dR}{dt} = k_{\mathrm{out}} \cdot \left[R_0 + (R_{\mathrm{min}} - R_0) \cdot \left(\frac{Cp(t)^{\gamma}}{Cp(t)^{\gamma} + \mathrm{IC}_{50}{}^{\gamma}} \right) \right] - k_{\mathrm{out}} \cdot R(t) \tag{7}$$

where, for either testosterone or inhibin, $R(t)$ is the measured concentration, R_0 is the base-line concentration, R_{min} is the minimum concentration when the input rate is maximally suppressed by nandrolone, k_{out} is the first-order elimination rate constant, $Cp(t)$ is the predicted plasma concentration for nandrolone (based on individual dosing and Bayesian pharmacokinetic parameter estimates) and IC_{50} is the concentration of nandrolone associated with 50% suppression of synthesis. The parameter γ was implemented as $\gamma = 1 + \theta$, to enable a comparison of the full model ($\gamma > 1$) and reduced model ($\gamma = 1$, $\theta = 0$) using the likelihood ratio test.

Alternatively, partial inhibition of the input rate can be modeled with an additional term expressing the fractional inhibition (I_{max}), as shown in equation 8.

$$\frac{dR}{dt} = k_{\mathrm{in}}^0 \cdot \left[1 - \frac{I_{\mathrm{max}} \cdot Cp(t)}{Cp(t) + \mathrm{IC}_{50}} \right] - k_{\mathrm{out}} \cdot R(t) \tag{8}$$

We used the parameterization shown in equation 7, because we were interested in estimating the base-line and maximally suppressed concentrations of testosterone and inhibin (and the interindividual variability in these parameters) directly. Based on the parameterization in equation 7, I_{max} is readily calculated as

$$I_{max} = 1 - \frac{R_{min}}{R_0} \quad (9)$$

The interindividual errors in the model parameters (R_o, R_{min}, k_{out}, IC_{50} and γ) were assumed to have a logarithmic-normal distribution, and the variance of the residual errors was assumed to be homoscedastic. As described for the pharmacokinetic analysis, a GAM was used to identify significant covariates, and NONMEM was used to develop the final pharmacodynamic model. All data are expressed as mean ± S.E.M.

Results

Volunteers randomized into the four groups were comparable in anthropometric and hormonal variables (table 1). There were no significant differences between groups in mean dehydroepiandrosterone sulfate, LH, FSH, prolactin, insulin-like growth factor-I, hemoglobin, urea or creatinine concentrations (data not shown), which were normal for all men.

Global statistical analysis. Considering all four groups, global statistical analysis demonstrated significant differences in the time course of plasma nandrolone concentrations (group, $\chi^2 = 84.6$, 3 dF, $P < .001$ by Wald test; group × time interaction, $\chi^2 = 643$, 66 dF, $P < .001$). These systematic differences were attributable to differences between different nandrolone esters (table 2). Similarly the time course of plasma testosterone concentrations varied significantly by group (group × time interaction, $\chi^2 = 266$, 66 dF, $P < .001$) due to effects of both ester and injection site (table 2). To adjust for the dominating effect of differences between esters, a global analysis conducted for the three groups receiving nandrolone decanoate (groups 2–4) demonstrated significant effects of injection site on plasma nandrolone levels, as well as effects of injection volume and site on plasma testosterone and inhibin levels (table 3).

Pharmacokinetic analysis. Plasma nandrolone concentrations reached higher and earlier peak concentrations and had a shorter mean residence time after injection of the phenylpropionate ester (group 1; $n = 7$), compared with the other three decanoate ester groups (fig. 1; tables 2 and 4). Analysis of the concentration data obtained from the two subjects who received i.v. nandrolone gave the following values: area under curve/unit dose = 1.3224×10^{-3} days/liter, mean residence time = 25.65 ± 5.22 min, volume of distribution = 11.46 ± 0.30 liters and systemic clearance = 31.52 ± 7.26 liters/hr. From the optimal triexponential curve fit, the following parameters were estimated: $A = 153.3 \pm 2.7$ nM, $\alpha = 0.4132 \pm 0.0030$ min^{-1}, $B = 8.8659 \pm 0.4575$ nM, $\beta = 0.0098 \pm 0.0022$ min^{-1}, $C = 0.9708 \pm 0.0066$ nM and $\gamma = 0.00043 \pm 0.00045$ min^{-1}. Based on the area under the curve estimates for these two subjects, the absolute bioavailability of nandrolone from i.m. injections of esters was significantly higher for nandrolone decanoate injected into gluteal muscle with a 1-ml volume (73%), compared with the other three groups (53–56%).

The final population pharmacokinetic model incorporated ester, site and volume of injection and height as significant covariates. Height was significantly superior to weight, body surface area or lean body mass as a covariate. The type of ester influenced the absorption profile of nandrolone, such that the phenylpropionate ester was best described by a one-compartment absorption model and the decanoate ester was best described by a two-compartment absorption model. This was implemented in the model with parameter P (table 5). The interpretation of this parameter was that, effectively, the total dose of the phenylpropionate ester is administered into the "fast" compartment characterized by the rapid absorption rate constant (k_1), whereas only ~14% of the total dose of the decanoate ester is administered into this compartment, with the remaining ~86% of the total dose being administered into the "slow" compartment characterized by a slower absorption rate constant (k_2). This basic difference in the profiles of the nandrolone concentration data is shown in figure 2. In figure 2, the individual in each of the four groups with the median mean absolute prediction error was selected

TABLE 1
Base-line anthropometric and endocrine variables

Ester	Nandrolone Phenylpropionate	Nandrolone Decanoate	Nandrolone Decanoate	Nandrolone Decanoate	P[e]
Injection					
Site	Gluteal	Gluteal	Gluteal	Deltoid	
Volume (ml)	4	4	1	1	
Dose (mg)	100	100	100	100	
Patients					
Number	7	6	5	5	
Age (yr)	26 ± 2	24 ± 1	24 ± 3	24 ± 2	0.842
Height (cm)	178 ± 2	177 ± 3	173 ± 5	171 ± 3	0.426
Weight (kg)	72.5 ± 3.1	69.7 ± 2.6	66.8 ± 4.4	72.1 ± 2.2	0.580
BSA (m^2)[a]	1.90 ± 0.05	1.86 ± 0.04	1.79 ± 0.08	1.84 ± 0.05	0.616
RBW (% ideal)[b]	101 ± 3	99 ± 4	98 ± 3	108 ± 2	0.165
BMI (kg/m^2)[c]	22.8 ± 0.7	22.4 ± 1.0	22.3 ± 0.6	24.7 ± 0.4	0.152
Total testosterone (nM)	17.6 ± 0.8	20.7 ± 2.5	17.9 ± 2.3	17.1 ± 1.4	0.498
Free testosterone (pM)	323 ± 38	384 ± 62	265 ± 26	298 ± 21	0.290
Estradiol (pM)	130 ± 18	120 ± 11	150 ± 34	83 ± 22	0.251
SHBG (nM)[d]	23.6 ± 2.0	25.8 ± 3.5	34.7 ± 0.7	26.9 ± 5.2	0.121

[a] BSA, bodt surface area.
[b] RBW, relative body weight.
[c] BMI, body mass index.
[d] SHBC, sex hormone binding globulin.
[e] From analysis of variance.

TABLE 2

Global statistical analysis of plasma nandrolone and testosterone after nandrolone ester injection by time course, ester, injection site and volume

Variable	Nandrolone			Testosterone		
	χ^2	dF	P	χ^2	dF	P
Ester	77.6	1	0	2.8	1	.093
Volume	1.7	1	.188	1.1	1	.291
Site	1.3	1	.261	0.2	1	.663
Time	665	22	0	596	22	<.001
Time × ester	609	22	0	168	22	<.001
Time × volume	10.7	22	.979	22.1	22	.455
Time × site	18.6	22	.671	64.2	22	<.001

TABLE 3

Global statistical analysis of plasma nandrolone, testosterone and inhibin after nandrolone decanoate injection by time course, injection site and volume

Variable	Nandrolone			Testosterone			Inhibin		
	χ^2	dF	P	χ^2	dF	P	χ^2	dF	P
Volume	2.9	1	.090	1.0	1	.315	0.5	1	.482
Site	2.1	1	.148	0.2	1	.681	1.3	1	.262
Time	466	22	0	455	22	0	148	22	<.001
Time × volume	21.5	22	.489	38.2	22	.017	26.5	22	.001
Time × site	49.7	22	.001	72.9	22	0	23.8	22	.003

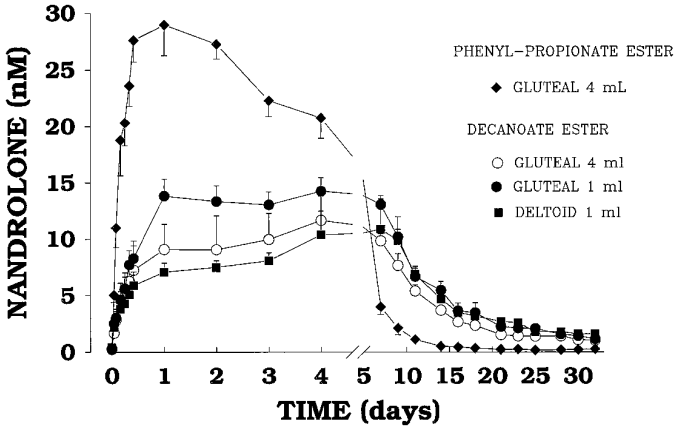

Fig. 1. Time course of plasma nandrolone concentrations in 23 healthy men over 32 days after i.m. injection of 100 mg of nandrolone phenylpropionate in 4 ml of arachis oil vehicle into the gluteal muscle (group 1) (♦) or injection of 100 mg of nandrolone decanoate into the gluteal muscle in 4 ml of arachis oil vehicle (group 2) (○), into the gluteal muscle in 1 ml of arachis oil vehicle (group 3) (●) or into the deltoid muscle in 1 ml of arachis oil vehicle (group 4) (■). Results are expressed as mean and S.E.M., unless the S.E. is smaller than symbol.

to represent the Bayesian predictions based on the individual pharmacokinetic parameters. In addition, the rate of absorption from the fast compartment (k_1) was greater for the deltoid muscle than the gluteal muscle.

A two-compartment disposition function performed significantly better than a one-compartment disposition function. Site and volume of injection were important covariates for the two parameters, with bioavailability as a component ($F{\cdot}A_1$ and $F{\cdot}A_2$). $F{\cdot}A_1$ was greater in the gluteal muscle, compared with the deltoid muscle, and $F{\cdot}A_2$ was greater with a smaller injection volume (1 ml *vs*. 4 ml). Although the slow hybrid rate constant λ_2 was difficult to estimate accurately, it describes the very slow terminal elimination phase (fig. 2) and significantly improved the logarithmic-likelihood objective function of the model, as exemplified by the improved fit of the two-compartment, compared with one-compartment, disposition function.

Pharmacodynamic analysis. Plasma testosterone concentrations were most rapidly and completely suppressed within the first week after injections of the phenylpropionate ester (fig. 3; tables 2 and 6), but this suppression was sustained for the shortest time. The duration of suppression was significantly longest after the gluteal 1-ml injection. Plasma testosterone concentrations returned to base line by day 13 after the phenylpropionate ester but required >20 days to return to base-line levels after the decanoate ester. Among the decanoate ester injections, both injection volume and site significantly influenced plasma testosterone concentrations (tables 3 and 6). Plasma inhibin levels after decanoate ester injections were suppressed to significantly lower nadir levels after 1-ml gluteal injection (fig. 3; table 6). Plasma inhibin was not assayed after nandrolone phenylpropionate (group 1) injection.

The GAM analysis detected no statistically significant covariates for the testosterone pharmacodynamic model (table 7). The inclusion of a parameter to estimate the nadir concentration of testosterone resulting from maximal suppression of testosterone synthesis by nandrolone and of a slope parameter describing the steepness of the relationship between the nandrolone concentration and the testosterone output rate significantly improved the model. Figure 4 shows the predicted testosterone concentrations for the same individuals as shown in figure 2. These predictions were calculated using the Bayesian estimates of the individual's nandrolone pharmacokinetic parameters and testosterone pharmacodynamic parameters.

No statistically significant covariates were detected in the GAM analysis for the inhibin pharmacodynamic model (figs. 5 and 6; table 7). Unlike testosterone, the inclusion of a parameter to estimate the nadir inhibin concentration did not improve the model, although a slope parameter did significantly improve the model fit. Figure 6 shows the pre-

TABLE 4
Pharmacokinetic variables

Variable (mean ± S.D.)	N-PP[a]	N-D	N-D	N-D	P[b]
Injection site	Gluteal	Gluteal	Gluteal	Deltoid	
Injection volume (ml)	4	4	1	1	
Dose (mg)	100	100	100	100	
Number of patients	7	6	5	5	
Time of peak (days)					
Empirical	1.0 ± 0.9	5.0 ± 3.2	2.4 ± 1.5	3.6 ± 2.3	.022
Fitted	1.1 ± 0.1	1.8 ± 0.3	1.6 ± 0.2	1.5 ± 0.2	<.001
Peak concentration (nM)					
Empirical	30.9 ± 5.8	13.5 ± 5.0	15.8 ± 2.8	12.4 ± 5.5	<.001
Fitted	32.5 ± 2.5	12.7 ± 1.1	16.1 ± 1.0	8.4 ± 0.5	<.001
Mean residence time (days)					
Empirical	4.5 ± 0.7	10.1 ± 1.5	10.0 ± 1.2	11.4 ± 1.1	<.001
Fitted	3.1 ± 0.2	9.5 ± 0.7	10.6 ± 0.9	16.9 ± 1.3	<.001
Half-time (days)					
Absorption	2.4 ± 0.1	7.0 ± 0.4	7.7 ± 0.6	12.0 ± 0.9	<.001
Clearance	0.25 ± 0.05	0.42 ± 0.11	0.34 ± 0.06	0.25 ± 0.05	.002
Duration ≥5 nM (days)	5.1 ± 2.0	9.4 ± 1.1	13.3 ± 2.9	9.0 ± 5.1	.002
Clearance (liters/day)	1359 ± 149	1355 ± 95	1041 ± 59	1421 ± 57	<.001
Area under the curve (nmol · day/liter)					
Empirical	155 ± 21	144 ± 30	193 ± 29	162 ± 34	.065
Fitted	181 ± 20	172 ± 12	224 ± 13	164 ± 7	<.001

[a] Esters are nandrolone decanoate (N-D) and nandrolone phenylpropionate (N-PP).
[b] From analysis of variance.

TABLE 5
Nandrolone population pharmacokinetic model

Parameter	Model[a]	C.V.[b]%
P	$1 - 0.863 \cdot$ ester flag	
k_1 (day^{-1})	$4.42 + 5.03 \cdot$ site flag	57
k_2 (day^{-1})	0.192	27
$F \cdot A_1$ (liter^{-1})	$0.000192 - 0.0000319 \cdot$ site flag	31
λ_1 (day^{-1})	$0.342 + 0.00297 \cdot$ [height (cm) − 174]	28
$F \cdot A_2$ (liter^{-1})	$0.00000197 + 0.00000248 \cdot$ volume flag	74
λ_2 (day^{-1})	0.000388	

[a] Ester flag, phenylproprionate = 0, decanoate = 1; site flag, gluteal = 0, deltoid = 1; volume flag, 4 ml = 0, 1 ml = 1.
[b] Coefficient of variation.

dicted inhibin concentrations for the same individuals as shown in figure 2. Plasma inhibin was not assayed after nandrolone phenylpropionate (group 1) injection. These predictions were calculated using the Bayesian estimates of the individual's nandrolone pharmacokinetic and inhibin pharmacodynamic parameters.

Discussion

The present study demonstrates that, in addition to the chemistry of the side-chain ester, both injection site and volume can systematically influence blood nandrolone levels after i.m. injection of nandrolone esters in an oil vehicle formulation. Corresponding to the patterns of blood nandrolone concentrations, pharmacodynamic indices reflecting androgen-induced inhibition of pituitary-testicular function, namely blood testosterone and inhibin concentrations, are also systematically influenced by these factors. Crucially, the mixed-effects pharmacodynamic modeling demonstrated that essentially all of the pharmacodynamic variability in plasma testosterone and inhibin concentrations was accounted for by the variability between esters and the site and volume of injection of the nandrolone injections. The present study extends knowledge of the clinical pharmacokinetics of nandrolone esters, which were reported in two previous studies concerning nandrolone decanoate kinetics in humans (Belkien *et al.*, 1985; Wijnand *et al.*, 1985); there are no reports of the pharmacokinetics of the phenylpropionate ester.

One feature of this study is the use of the population pharmacokinetic approach to integrating pharmacokinetic and pharmacodynamic data. Whereas the global statistical analysis indicates the significance of some key variables, a structural model allows more physiological interpretation of the findings, especially identifying the relationships between the pharmacokinetic and pharmacodynamic effects. We have used an indirect response model (Dayneka *et al.*, 1993), which allows for more physiologically meaningful models, more realistic interpretation of derived estimates and statistically valid testing for categorical covariables, while more efficiently using all of the experimental data (Jusko and Ko, 1994). The indirect physiological model-based approach also readily allows incorporation, into a general model, of data from different subpopulations where the kinetics and dynamics had differing shapes for the effective dose-response relationships. It is a striking validation of the model-based estimates that the nadir concentrations of testosterone and inhibin correspond very accurately to the known concentrations of these hormones in castrated men (1–3 nM and 0 pg/ml, respectively) (McLachlan *et al.*, 1990; Handelsman, 1994).

An interesting feature of this analysis of testosterone suppression is that the first-order rate constant for the response compartment (k_{out}) does not correspond to the metabolic clearance rate for testosterone. Using either bolus injection or steady-state infusion, the metabolic clearance rate for testosterone in men is ~540 liters/m^2/day (Gandy, 1977) and the volume of distribution is 10 to 20 liters, which is similar to the 11.5 liters estimated in this study for nandrolone, a molecule almost identical to testosterone. These estimates would indicate a k_{out} value of ~50 day^{-1}, whereas our observed estimate for k_{out} was 0.708 day^{-1}; the latter is consistent with a much slower rate (half-time, ~1 day). This discrepancy is attributable to the fact that the overall kinet-

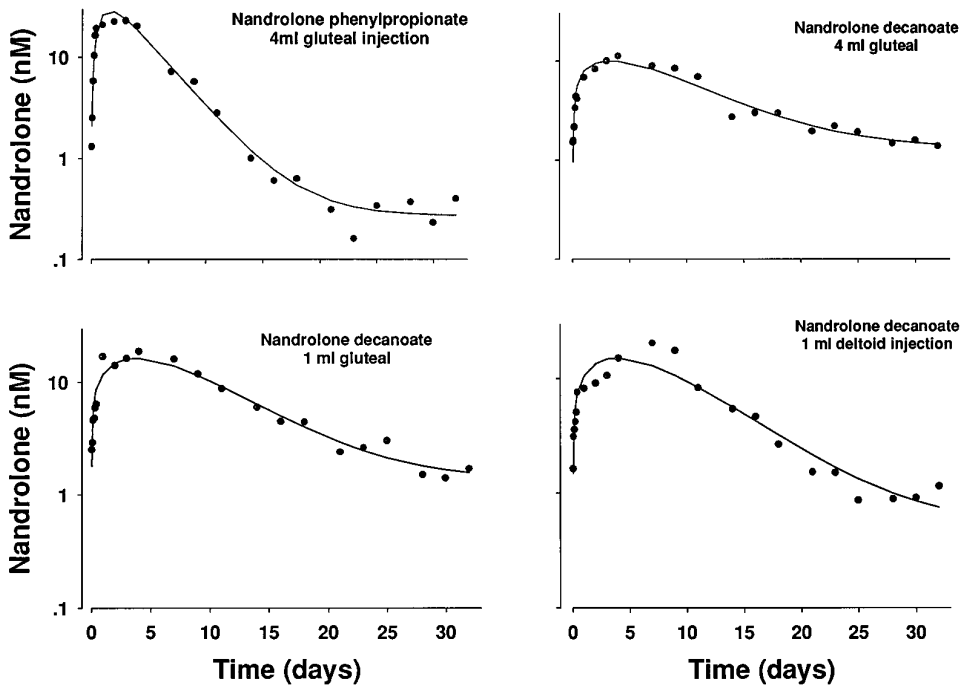

Fig. 2. Observed and model-predicted time course of plasma nandrolone concentrations in four healthy men over 32 days after i.m. injection of 100 mg of nandrolone ester. The four men were selected from each of the treatment groups according to the median predicted error in nandrolone concentrations, so that they were most representative of that group. Note the logarithmic vertical scale. ●, observed data; ——, individual Bayesian predictions.

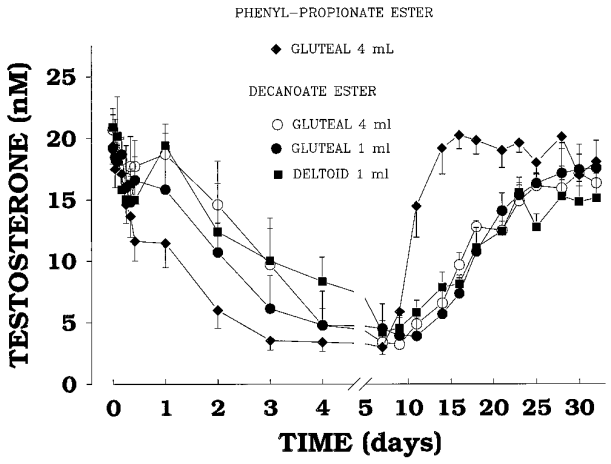

Fig. 3. Time course of plasma testosterone concentrations in 23 healthy men over 32 days after i.m. injection of 100 mg of nandrolone phenylpropionate in 4 ml of arachis oil vehicle into the gluteal muscle (group 1) (◆) or injection of 100 mg of nandrolone decanoate into the gluteal muscle in 4 ml of arachis oil vehicle (group 2) (○), into the gluteal muscle in 1 ml of arachis oil vehicle (group 3) (●) or into the deltoid muscle in 1 ml of arachis oil vehicle (group 4) (■). Results expressed as mean and S.E.M., unless the S.E. is smaller than the symbol.

ics of suppression of testosterone are dominated by the slow negative feedback system, rather than the much faster metabolic clearance of testosterone. This negative feedback is mediated *via* inhibition of pulsatile gonadotropin-releasing hormone secretion from hypothalamic neurons into the pituitary portal system and then pituitary LH secretion from gonadotropes. For example, a highly potent and specific gonadotropin-releasing hormone antagonist that causes immediate cessation of gonadotropin-releasing hormone action leads to castrate testosterone concentrations within 12 hr (Behre *et al.*, 1992), compared with 5 to 10 days in this study. This illustrates the need for physiological insight when interpreting indirect pharmacodynamic models because, in this instance, the relationship between circulating nandrolone concentration and the input function to the model may itself be indirect. Our paradigm exemplifies a paradigm where the k_{out} parameter may accurately predict the time course of overall behavior of a system without corresponding to the metabolic clearance rate of the drug or the pharmacodynamic endpoint under study.

In the present study we have used specific radioimmunoassays to measure the nandrolone, testosterone and inhibin concentrations, with the latter two representing effective markers of endogenous pituitary gonadotropin (LH and FSH, respectively) secretion. This reflects the physiological fact that pituitary LH acts exclusively upon testicular Leydig cells, due to their unique expression of cell surface membrane LH receptors. In healthy men, virtually all circulating testosterone originates from Leydig cells, with an absolute requirement for trophic influence from LH derived from the bloodstream. Similarly, pituitary FSH acts exclusively upon testicular Sertoli cells, which uniquely express FSH receptors on their cell surface membranes, and virtually all circulating immunoreactive inhibin originates from the gonads (Burger, 1992). As a result, blood levels of these two hormones are useful integrated bioassay indicators of endogenous pituitary gonadotropin secretion, as reflected by the testicular hormonal response to ambient blood LH and FSH levels. In the present study, these two pharmacodynamic indices showed physiologically meaningful distinctions between the esters and the effects of injection site and volume.

Variations in side-chain ester chemistry are important in the pharmacokinetics of androgen esters in oil vehicle (Behre *et al.*, 1990). Experimental studies suggest that absorption rates are predicted by the oil/water partition coefficients (or hydrophobicity) and that the oil vehicle is absorbed more slowly than the androgen ester (Tanaka *et al.*, 1974). In humans, the very short propionate (three-carbon aliphatic) ester of testosterone has distinctly shorter duration of action than esters with longer (seven- or eight-carbon) side-chains (Nieschlag *et al.*, 1976; Schulte-Beerbuhl and Nieschlag,

TABLE 6
Pharmacodynamic variables

Variable (mean ± S.D.)	ND-PP[a]	ND-D	ND-D	ND-D	P[b]
Injection site	Gluteal	Gluteal	Gluteal	Deltoid	
Injection volume (ml)	4	4	1	1	
Dose (mg)	100	100	100	100	
Number of patients	7	6	5	5	
Testosterone					
Base-line concentration (nM)	17.6 ± 0.8	20.7 ± 2.5	17.9 ± 2.3	17.1 ± 1.4	.498
Nadir concentration (nM)	2.3 ± 1.4	2.4 ± 1.1	1.7 ± 0.7	3.2 ± 1.3	.295
Time of nadir (days)	5.2 ± 2.1	9.2 ± 2.6	8.4 ± 3.0	7.2 ± 2.0	.060
Net secretion (nM · days)	464 ± 89	348 ± 51	294 ± 65	341 ± 64	.004
Duration of suppressed testosterone (days)[c]	5.8 ± 3.1	11.0 ± 8.2	17.5 ± 5.0	14.9 ± 4.7	.010
Inhibin					
Base-line concentration (μg/liter)	NA[d]	137 ± 29	164 ± 23	189 ± 47	.559
Nadir concentration (μg/liter)	NA	81 ± 55	50 ± 11	79 ± 65	.009
Time of nadir (days)	NA	4.5 ± 2.7	4.0 ± 2.7	3.0 ± 2.2	.291
Net secretion (μg · days/liter)	NA	4052 ± 2735	2429 ± 883	3502 ± 2650	.465
Duration of suppressed inhibin (days)[c]	NA	0 ± 0	6.0 ± 9.7	5.4 ± 10.0	.096

[a] ND-PP, nandrolone phenylpropionate; ND-D, nandrolone decanoate.
[b] From parametric analysis of variance for testosterone and from nonparametric analysis (Kruskal-Wallis test) for inhibin, due to data skewing reflecting high between-subject variability.
[c] Suppressed levels defined as <10 nM for testosterone and <50% base line for inhibin.
[d] NA, not assayed.

TABLE 7
Population pharmacodynamic models for testosterone and inhibin

Parameter[a]	Testosterone[b]		Inhibin	
	Population Mean	Population Variability (%)	Population Mean	Population Variability (%)
Base line (R_0)	18.5 ± 0.7	14	153 ± 17	51
Nadir (R_{min})	1.9 ± 0.5		0	
k_{out} (day^{-1})	0.708 ± 0.05	87	0.189 ± 0.042	
Nandrolone IC_{50} (nM)	2.88 ± 0.51	38	7.49 ± 0.83	
Slope parameter (γ)	2.28 ± 0.22	42	1.98 ± 0.48	

[a] For details of parameterization, see equation 7 in "Materials and Methods."
[b] Units for baseline and nadir are nM (testosterone) or pg/ml(inhibin).

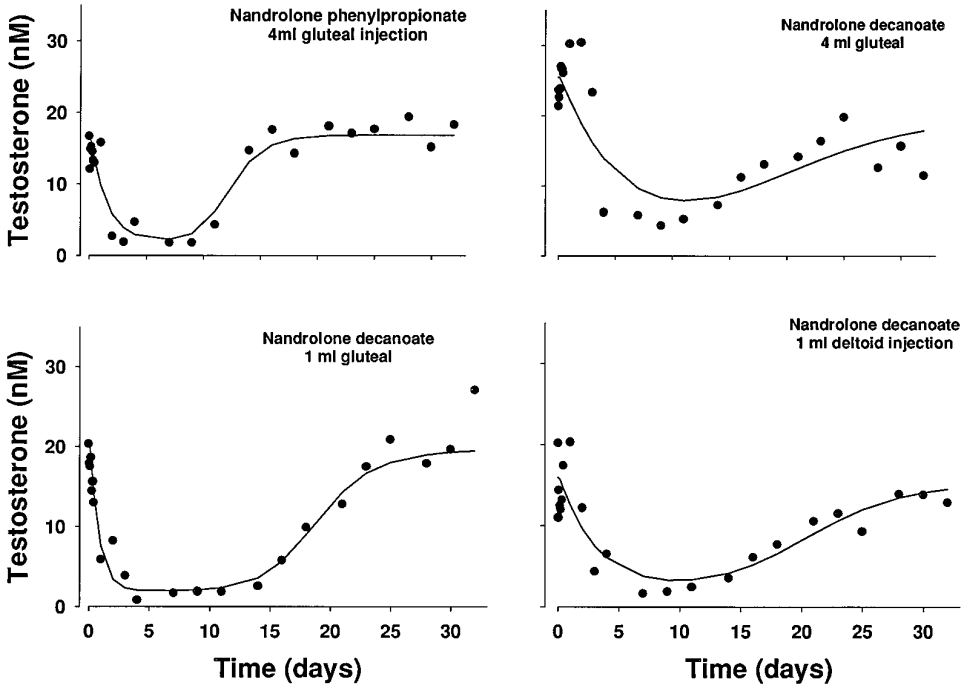

Fig. 4. Observed and model-predicted time course of plasma testosterone concentrations in four healthy men over 32 days after i.m. injection of 100 mg of nandrolone ester. The four men were the same individuals as illustrated in figure 2, who were originally selected from each of the treatment groups according to the median predicted error of nandrolone concentrations, so that they were most representative of that group. ●, observed data; ——, individual Bayesian predictions.

1980; Schurmeyer and Nieschlag, 1984; Belkien *et al.*, 1985; Fujioka *et al.*, 1986). More subtle changes in side-chain ester structure have proven ineffective in altering human clinical pharmacokinetics, because substitution of a linear aliphatic side-chain of seven carbons (enanthate) with either a saturated, cyclized, seven-carbon aliphatic chain (cyclohexan-

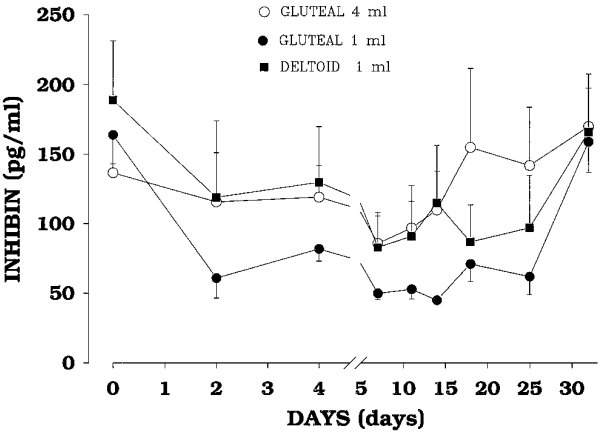

Fig. 5. Time course of plasma inhibin concentrations in 23 healthy men over 32 days after i.m. injection of 100 mg of nandrolone decanoate into the gluteal muscle in 4 ml of arachis oil vehicle (group 2) (○), into the gluteal muscle in 1 ml of arachis oil vehicle (group 3) (●) or into the deltoid muscle in 1 ml of arachis oil vehicle (group 4) (■). Results are expressed as mean and S.E.M. unless the S.E. is smaller than the symbol.

ecarboxylate) (Schurmeyer and Nieschlag, 1984) or a linear, aliphatic, eight-carbon chain (cypionate) (Schulte-Beerbuhl and Nieschlag, 1980) resulted in virtually unchanged kinetics. Wider variation in ester side-chain chemistry to include greater chain length and/or aromatic ring structures is a more effective determinant of ester pharmacokinetics, because nandrolone hexoxyphenylpropionate ester (aromatic ring with 18 carbons) had far better depot properties, with a prolonged and retarded release profile, compared with the decanoate (aliphatic chain with 10 carbons) (Belkien *et al.*, 1985). The present study indicates that a side-chain ester consisting of a 10-carbon aliphatic chain has better depot properties than a nine-carbon chain including an aromatic ring. Because the vehicle (arachis oil) was unchanged during this study and because of the experimental observation that the oil vehicle influences local reaction to the oil injection (Brown *et al.*, 1944), as well as androgen ester pharmacology (Ballard, 1980; Al-Hindawi *et al.*, 1986), the present conclusions may be extrapolated to other vegetable oil injection vehicles only with caution.

Injection technique, including injection site, volume and concentration, as well as the nature of the vehicle, could theoretically be important for androgen ester release rate. Injection site may be important because of differences in tissue composition (Cockshott *et al.*, 1982) and blood flow (Bederka *et al.*, 1971); indeed, i.m. oil-based injections may more accurately be termed intermuscular (Ballard, 1968) or intralipomatous (Cockshott *et al.*, 1982). The former reflects the tendency of oil vehicle to distribute along intermuscular fascial planes (Ballard, 1968), whereas the latter depends upon the amount of fat at the injection site (including systematic gender differences) (Modderman *et al.*, 1983) together with needle geometry and anatomy of the injection depot. Intralipomatous deposition of injections with a larger vehicle volume may explain the slower release kinetics of nandrolone decanoate in the gluteal region, as well as the differences from the deltoid site, which has a lower fat content. The higher blood flow in the deltoid, compared with the gluteal, muscle (Evans *et al.*, 1975) may also be important. Analogous site-dependent differences in absorption rate and physiological effects have been described for a variety of drugs in aqueous solution (Greenblatt and Koch-Weser, 1976). To our knowledge, there are no previous reports examining the systemic pharmacokinetic and pharmacodynamic effects of injection site and volume for androgen esters in oil vehicle in men.

One possible clinical impact of these observations may lie in recent observations of differences between population groups in the efficacy of regular i.m. injections of testosterone enanthate in an oil vehicle to suppress testicular function for male contraception (World Health Organization Task Force on Methods for the Regulation of Male Fertility, 1990). In

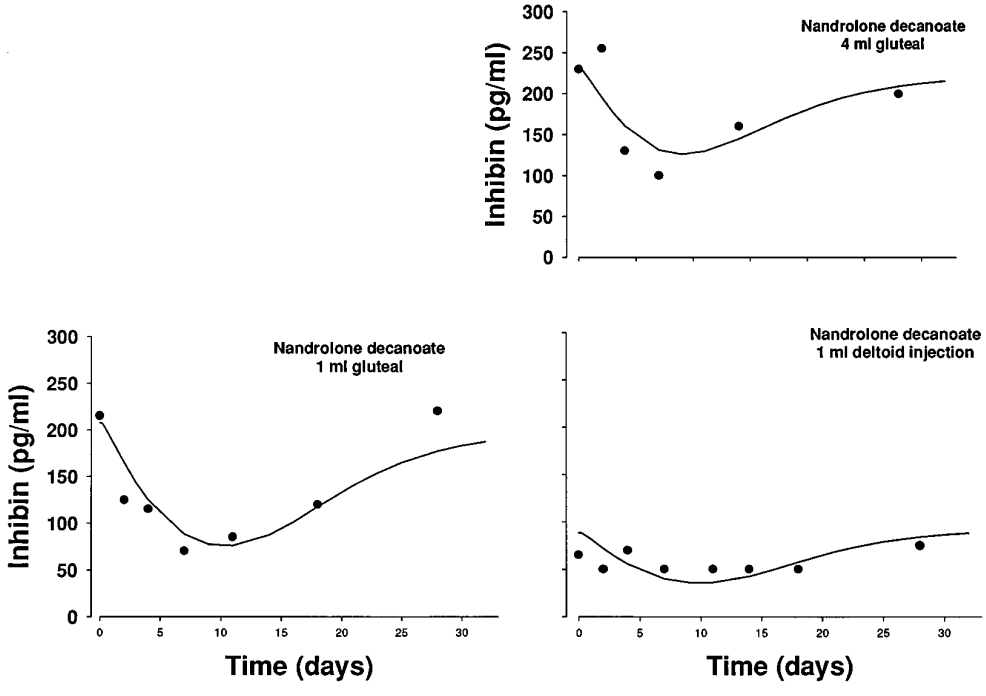

Fig. 6. Observed and model-predicted time course of plasma inhibin concentrations in three healthy men over 32 days after i.m. injection of 100 mg of nandrolone decanoate. No inhibin data were available for the group that received nandrolone phenylpropionate injections. The three men were selected from three of the four treatment groups according to the median predicted error, so that they were most representative of that group. ●, observed data; ——, individual Bayesian predictions.

that and related (World Health Organization Task Force on Methods for the Regulation of Male Fertility, 1993) studies, interethnic differences in susceptibility to androgen-induced azoospermia were not due to differences in overall body size or related differences (Handelsman *et al.*, 1995). Evaluation of the possibility of ethnopharmacological differences, however, required a greater understanding of the rate-determining mechanisms of androgen release from androgen ester depots in oil vehicles. The present findings suggest that differences in absorption of androgen esters may contribute to such interethnic differences through possible local mechanical factors (*e.g.,* exercise, compression and muscle and fat mass) at the injection site, and this issue warrants further study. Analogous variations in the pharmacokinetics of steroid esters have been reported among women from different countries using long-acting contraceptive steroids (Garza-Flores, 1994), although no explanation has been advanced. Further analysis of the present observations may facilitate such ethnopharmacological studies, as well as clinical applications of androgen esters in oil vehicle formulations.

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REVIEW

Pharmacology of anabolic steroids

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Athletes and bodybuilders have recognized for several decades that the use of anabolic steroids can promote muscle growth and strength but it is only relatively recently that these agents are being revisited for clinical purposes. Anabolic steroids are being considered for the treatment of cachexia associated with chronic disease states, and to address loss of muscle mass in the elderly, but nevertheless their efficacy still needs to be demonstrated in terms of improved physical function and quality of life. In sport, these agents are performance enhancers, this being particularly apparent in women, although there is a high risk of virilization despite the favourable myotrophic–androgenic dissociation that many xenobiotic steroids confer. Modulation of androgen receptor expression appears to be key to partial dissociation, with consideration of both intracellular steroid metabolism and the topology of the bound androgen receptor interacting with co-activators. An anticatabolic effect, by interfering with glucocorticoid receptor expression, remains an attractive hypothesis. Behavioural changes by non-genomic and genomic pathways probably help motivate training. Anabolic steroids continue to be the most common adverse finding in sport and, although apparently rare, designer steroids have been synthesized in an attempt to circumvent the dope test. Doping with anabolic steroids can result in damage to health, as recorded meticulously in the former German Democratic Republic. Even so, it is important not to exaggerate the medical risks associated with their administration for sporting or bodybuilding purposes but to emphasize to users that an attitude of personal invulnerability to their adverse effects is certainly misguided.
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Keywords: anabolic steroids; clinical; designer; health; mechanism; performance; receptor; SARMs; sport

Abbreviations: AF, activation function; BALCO, Bay Area Laboratory Co-operative; DHEA, dehydroepiandrosterone; DHT, 5α-dihydrotestosterone; FDA, Food and Drug Administration; Hsp, heat-shock protein; LC–MS/MS, liquid chromatography–mass spectrometry/mass spectrometry; MENT, 7α-methyl-19-nortestosterone; SARM, selective androgen receptor modulator; THG, tetrahydrogestrinone; UCLA, University of California, Los Angeles; WADA, World Anti-Doping Agency

Introduction

Androgens

Androgens exert their effects in many parts of the body, including reproductive tissues, muscle, bone, hair follicles in the skin, the liver and kidneys, and the haematopoietic, immune and central nervous systems (Mooradian *et al*., 1987). The androgenic effects of these hormones can be generally considered as those associated with masculanization and the anabolic effects as those associated with protein building in skeletal muscle and bone.

In the male foetus, androgens stimulate the development of the Wolffian ducts (epididymis, vas deferens, the seminal vesicles and ejaculatory duct) and the male external genitalia (penis, urethra and scrotum) (Wilson *et al*., 1981). During puberty, the androgenic effects resulting from increased testicular steroidogenesis are manifested by growth of the testes, external genitalia and the male accessory reproductive glands (prostate, seminal vesicles and bulbourethral), and secretory activity begins. Further, the secondary sexual characteristics manifested during puberty can be divided into those that are a result of androgenic and anabolic effects. The androgenic effects are the enlargement of the larynx causing a deepening of the voice, the growth of terminal hair (in the pubic, axillary and facial regions; in other regions such growth depends on a number of factors), an increase in sebaceous gland activity (can lead to acne), and CNS effects (libido and increased aggression). Anabolic effects are the growth of skeletal muscle and bone, the stimulation of linear growth eventually ceasing due to the closure of the epiphysis. In men, androgens are essential for sustaining reproductive function, and they play an important role in maintaining skeletal muscle and bone, cognitive function and a sense of well-being.

The most important androgen secreted is testosterone; in the eugonadal man, the Leydig cells in the testes produce

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~95% of the testosterone in the body. The ovaries and the adrenal glands (in both sexes) produce very little testosterone but secrete weaker androgens; in particular, dehydroepiandrosterone (DHEA; and its sulpho-conjugate) and androstenedione are of physiological importance in the women, not least because they can undergo peripheral conversion to more potent androgens, for example to testosterone and 5α-dihydrotestosterone (DHT). Another weaker endogenous androgen, androstenediol, also binds to oestrogen receptors.

The effects of androgens are modulated at cellular level by the steroid-converting enzymes within the particular target tissue (Figure 1). In reproductive target tissues, testosterone can be considered to be a prohormone, being readily converted by 5α-reductase to the more potent androgen DHT. In other tissues, such as adipose tissue and parts of the brain, testosterone is converted by aromatase to the oestrogen, oestradiol. In bone, the mechanism of action of the anabolism of androgens has not been entirely elucidated but both a direct effect of testosterone and a mediated effect by aromatization to oestradiol are important (Orwoll, 1996; Zitzmann and Nieschlag, 2004). In the human skeletal muscle (collected less than 12 h post-mortem), 5α-reductase activity (either type 1 or 2) is not detectable (Thigpen *et al.*, 1993), so testosterone itself is chiefly binding to the androgen receptor (as supported also by a number of animal studies, mainly in the rat). Aromatase expression and activity is significant in human skeletal muscle (Larionov *et al.*, 2003) but whether the conversion of androgens to oestrogens within this tissue is physiologically important for mediating some of the myotrophic effect of androgens is yet to be determined.

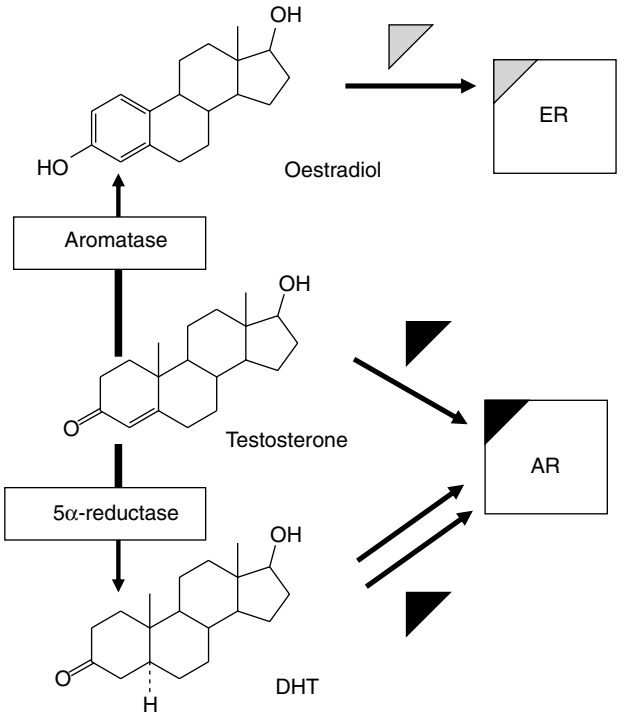


Figure 1 Testosterone can bind directly with the androgen receptor (AR). In target tissues where intracellular enzymes are present, the action of testosterone is mediated by metabolism. Testosterone is irreversibly converted by the enzyme 5α-reductase to 5α-dihydrotestosterone (DHT), which binds with greater affinity to the androgen receptor (AR), or by aromatase to oestradiol, which binds to the oestrogen receptor (ER). Testosterone and DHT can be also converted to weaker androgens (not displayed), again being dependent on whether the target tissue has the necessary enzyme activity, e.g., 3α-hydroxysteroid dehydrogenase, 17β-hydroxysteroid dehydrogenase.

Modulation of the effects of androgens may also occur at the molecular level due to differences in the distribution of androgen receptor coregulators in various tissues, these coregulators being proteins that affect the transcriptional activity of the androgen receptor (Heinlein and Chang, 2002b; Wolf and Obendorf, 2004). This is a developing field and the comparative importance of many of these coregulators is yet to be established for any particular cell type, let alone their relative *in vivo* importance in examining tissue differences in androgen action. It is envisaged that genetic manipulation of the mouse will assist in elucidating their physiological relevance.

With structural modifications to testosterone, the anabolic effects of androgens can be enhanced but, even so, these cannot be divorced entirely from their androgenic effects. Hence, a more accurate term for anabolic steroids is anabolic–androgenic steroids, but, for simplicity, the shorter term is used within this paper. The disassociation of anabolic from androgenic effects can be at cellular level, depending on the intracellular metabolism of the anabolic steroid in different tissues, with the activity of 5α-reductase being particularly important (see the section 'Intracellular metabolism and the myotrophic–androgenic index'). An appealing hypothesis is that anabolic–androgenic dissociation can also occur as a result of anabolic steroids inducing specific conformational changes of the androgen receptor complex, which then affects subsequent interaction with various coregulators in different tissues (see the section 'Androgen receptor expression and the importance of coregulators'). There is little data, as yet, to support such a hypothesis, but it is known that the androgen co-activator FHL2 is expressed predominantly in the heart (Muller *et al.*, 2000; Wolf and Obendorf, 2004), and it is possible that a number of other androgen receptor coregulators could be tissue specific. How an anabolic steroid may affect androgen receptor conformation and interaction with particular coregulators is of obvious interest, as such knowledge may eventually offer an additional mechanism for anabolic–androgenic dissociation.

The development of nonsteroidal selective androgen receptor modulators (SARMs) may offer better dissociation of biological effects than anabolic steroids and possibly even permit the therapeutic targeting of specific tissues and organs. Potential therapeutic modalities could then be specific agonists for restoration of fat-free muscle mass and strength in those with chronic illnesses such as HIV and specific antagonists for the treatment of prostate cancer in men or hirsutism in women (Wolf and Obendorf, 2004; Bhasin *et al.*, 2006). In anticipation of the potential of such agonists for performance enhancement in sport, SARMs have been added to the World Anti-Doping Agency's (WADA's) 2008 list of prohibited substances in sport, despite none yet being available on the market.

Control of anabolic steroids

Anabolic steroids are controlled substances in several countries, including Australia, Argentina, Brazil, Canada, the United Kingdom and the United States. Even so, there is a readily available supply of steroids worldwide for non-medicinal purposes, because, in most countries, anabolic steroids can be sold legally without a prescription (Hermansson, 2002; Cramer, 2005). Thus, many foreign distributors do not violate the laws of their own country when they sell these substances to customers overseas via the Internet and by e-mail orders. The majority of the hormone products in the European market come from countries within the European Union and Russia, but also sometimes from Thailand, Turkey, Egypt, India and Pakistan (Hermansson, 2002). In the United States, significant quantities of anabolic steroids come from Mexico, as well as other countries such as Russia, Romania and Greece (Cramer, 2005).

In the United Kingdom, anabolic steroids are controlled under Schedule IV Part 2 of the Misuse of Drugs Act; the Act includes most of the anabolic steroids, together with clenbuterol (adrenoreceptor stimulant) and human growth hormone. There is no restriction on the possession of these substances when they are part of a medicinal product and are for self-administration. However, prosecutions of intent to supply have been made of individuals found in possession of large quantities of these substances without a prescription for them. A Home Office licence is required for importation and exportation of anabolic steroids, except in cases of small quantities for legitimate purposes.

As to doping control in human sport, the International Olympic Committee (IOC) Medical Commission introduced anabolic steroids as a banned class in 1974 (Kicman and Gower, 2003b). The name of this banned class was amended to anabolic agents in the 1990s to incorporate out-of-competition testing for clenbuterol and other β_2-agonists, which are also considered to have anabolic activity. In 1999, WADA was set up as a foundation under the initiative of the IOC with the support and participation of intergovernmental organizations, governments, public authorities, and other public and private bodies fighting against doping in human sport. Under WADA, the rules and technical documents concerning anabolic steroids (and other drugs) are constantly evolving and for up to date information the reader is strongly advised to access the WADA web site (http://www.wada-ama.org/en/).

Misuse of anabolic steroids in sport and society

The use of anabolic steroids for cosmetic benefits among both adults and adolescents in society may be incorrectly regarded as a comparatively harmless pharmacological manipulation that can aid the development of bulging muscles and a well-toned figure. Surveys of anabolic steroid abuse by gymnasia users found that, overall, around 5% were using such drugs (Korkia and Stimson, 1993), whereas among people attending gyms equipped for competitive bodybuilding, the proportion of current or previous users was around 25–50% (Lenehan *et al.*, 1996; Korkia and Stimson, 1997). Nevertheless, it is difficult to estimate the true number of anabolic steroid users in the whole of the United Kingdom but these drugs are used on a nationwide basis, as discussed in depth by the report from the British Medical Association (BMA, 2002). Similar surveys indicate a high prevalence of use in the United States (Yesalis *et al.*, 1993, 1997; Yesalis and Bahrke, 2000).

For drug control in sport, anabolic steroids are regarded (correctly) as performance enhancers, as well as harmful to health. Of the 198 143 urine samples analysed in 2006 by 34 WADA-accredited laboratories, 4332 (2%) were found to contain a prohibited substance ('A-sample'), of which 1966 (45% of all the adverse findings) were positive for anabolic steroids. Comparison of the adverse findings for worldwide testing for over a decade show that there has been little change year after year, the most common steroids being testosterone, nandrolone, stanozolol and methandienone. Testosterone has an unfavourable anabolic–androgenic dissociation compared with other anabolic steroids, but it is more difficult to prove its administration, as it is also produced endogenously. Some consider that the WADA statistics do not reflect the real extent of doping with anabolic steroids, particularly within top-level athletics but few would dispute that the urge to succeed and the rewards of success, both financial and otherwise, have provided powerful incentives to some competitors to look for every possible means of improving their performance, despite the risk of denunciation and penalties.

Chemical structures and activity

Common anabolic steroids

Some of the structural modifications that have been introduced into the testosterone in an attempt to maximize the anabolic effect and minimize the androgenic are shown in Figure 2, and examples of anabolic steroids are given in Figure 3. Many of these steroids have been withdrawn as licensed products in numerous countries worldwide but they continue to be available as pharmaceutical preparations in others, for example, methandienone, methyltestosterone, oxandrolone and stanozolol. The only preparations currently available as licensed products for human use within the United Kingdom are testosterone and its esters, nandrolone (as the decanoate ester), mesterolone and oxymetholone (named patient basis only). Boldenone and trenbolone are restricted to veterinary purposes only in some countries, but, nonetheless, sports competitors and bodybuilders have been known to administer these anabolic steroids.

Oral activity can be conferred by substitution of the 17α-H on the steroid nucleus with a methyl or ethyl group to make the 17α-alkylated anabolic steroids. Alkyl substitution prevents deactivation of the steroid by first-pass metabolism by sterically hindering oxidation of the 17β-hydroxyl group. A methyl group attached to C-1 can also confer oral activity, as in methenolone or mesterolone, but these two anabolic steroids are considered to be relatively weak in pharmacological activity.

Parenteral preparations do not require a 17α-alkyl group but usually the 17β-hydroxyl group is esterified with an acid moiety (van der Vies, 1993) to prevent rapid absorption from

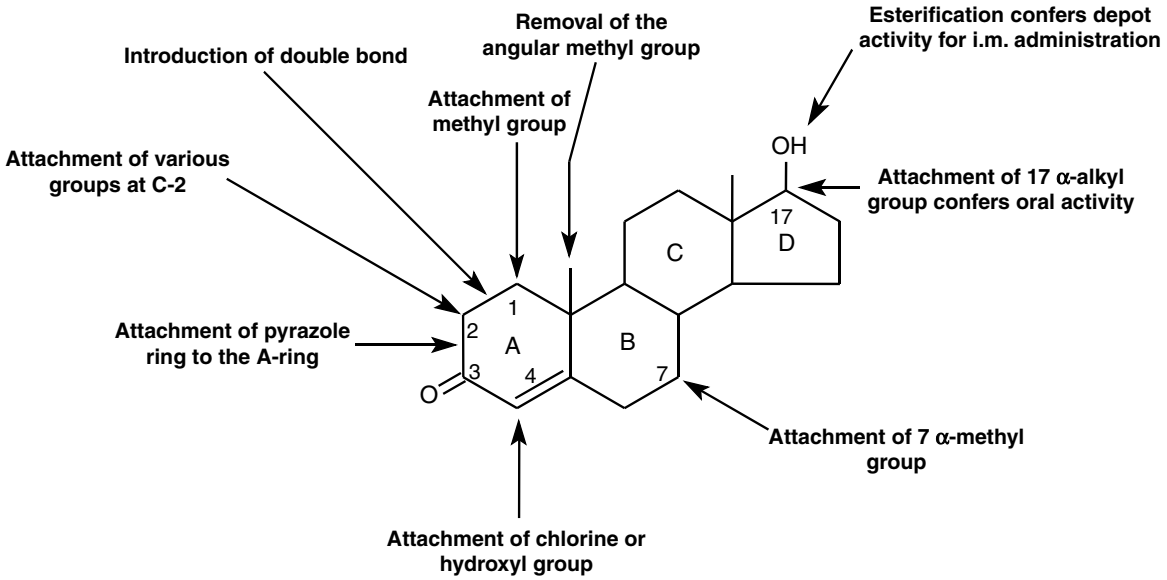


Figure 2 Structural modifications to the A- and B-rings of testosterone that increase anabolic activity; substitution at C-17 confers oral or depot activity (i.m.). Figure from Kicman and Gower (2003b), a commissioned article by the Analytical Investigations Standing Committee, reproduced with permission from the Association of Clinical Biochemists.

the oily vehicle, usually arachis oil plus a small amount of benzyl alcohol. Once in the circulation, hydrolysis rapidly occurs by the action of blood esterases to yield the active compound. The esters include cyclohexylpropionate, decanoate, laurate and phenylpropionate for nandrolone; acetate, cypionate, decanoate, enanthate, isocaproate, phenylpropionate, propionate and undecanoate for testosterone, undecylenate for boldenone and acetate for trenbolone. The mechanism of action of the nandrolone esters and other anabolic steroids, and the effect of drug delivery systems on their biological activity have been studied by van der Vies (1993). The duration of action of the esters depends upon the rate of absorption from the site of administration. This is dependent on the chain length of the acid moiety and also the formulation, being related to the partition coefficient of the derivatives between the oil used in the formulation and plasma. In general, the longer the chain length, the more slowly the preparation is released into circulation, thus prolonging the duration of action. Furthermore, testosterone undecanoate is also orally active, the 11 carbon chain ester making the molecule so lipophilic that its route of absorption is partially shifted from the hepatic portal vein to the lymph system, thus bypassing first-pass metabolism to some extent, it being released into the circulation via the thoracic duct (Coert *et al*., 1975; Horst *et al*., 1976).

Non-pharmaceutical water-based testosterone suspensions for injection are advertised on bodybuilding web sites and cheats in sport may find these attractive as, in theory, these should be relatively short acting. Non-pharmaceutical-based preparations, whether oil or water based, may be a particular hazard to health as the contents may not have been prepared under sterile conditions.

Transdermal formulations are invariably testosterone based, legitimately designed for replacement therapy, and include the 'patch' and hydroalcoholic gels, to be applied on a daily basis. Other short-acting testosterone preparations include those that are designed to be administered by the sublingual or buccal route. Such short-acting formulations are of particular concern in sport, as the exogenous source of testosterone is rapidly eliminated following cessation of treatment. Increased out-of-competition testing helps to combat the cheat who is using short-acting preparations and ceasing administration prior to competition in anticipation of testing. It is of interest that an illicit preparation called 'The Cream' was designed for transdermal application (see the section 'Designer steroids').

Steroid dietary supplements

A current cause for concern is the recent manufacture of analogues of established anabolic steroids to tap into the bodybuilding market. To avoid the statutory controls of countries regarding the manufacture and supply of drugs, these compounds are often widely marketed as nutritional/dietary supplements, examples being DHEA, androstenedione, androstenediol, and their 19-nor equivalents (these steroids are prohormones), and analogues of testosterone and stanozolol called 1-testosterone and prostanozolol, respectively (Figure 4). It is a consequence of their widespread availability that a minority of athletes will also use these steroids in an attempt to improve sporting performance, and because they are structurally related to mainstream anabolic steroids, sports antidoping laboratories are made to incorporate such compounds into their drug screens under the WADA rules. These steroids are supplied for oral administration, and are therefore subject to first-pass metabolism, a very important factor as to the extent the steroid is deactivated or converted to a more active form. Some of the putative metabolites of dietary supplements have been identified by mass spectrometry, but not by other

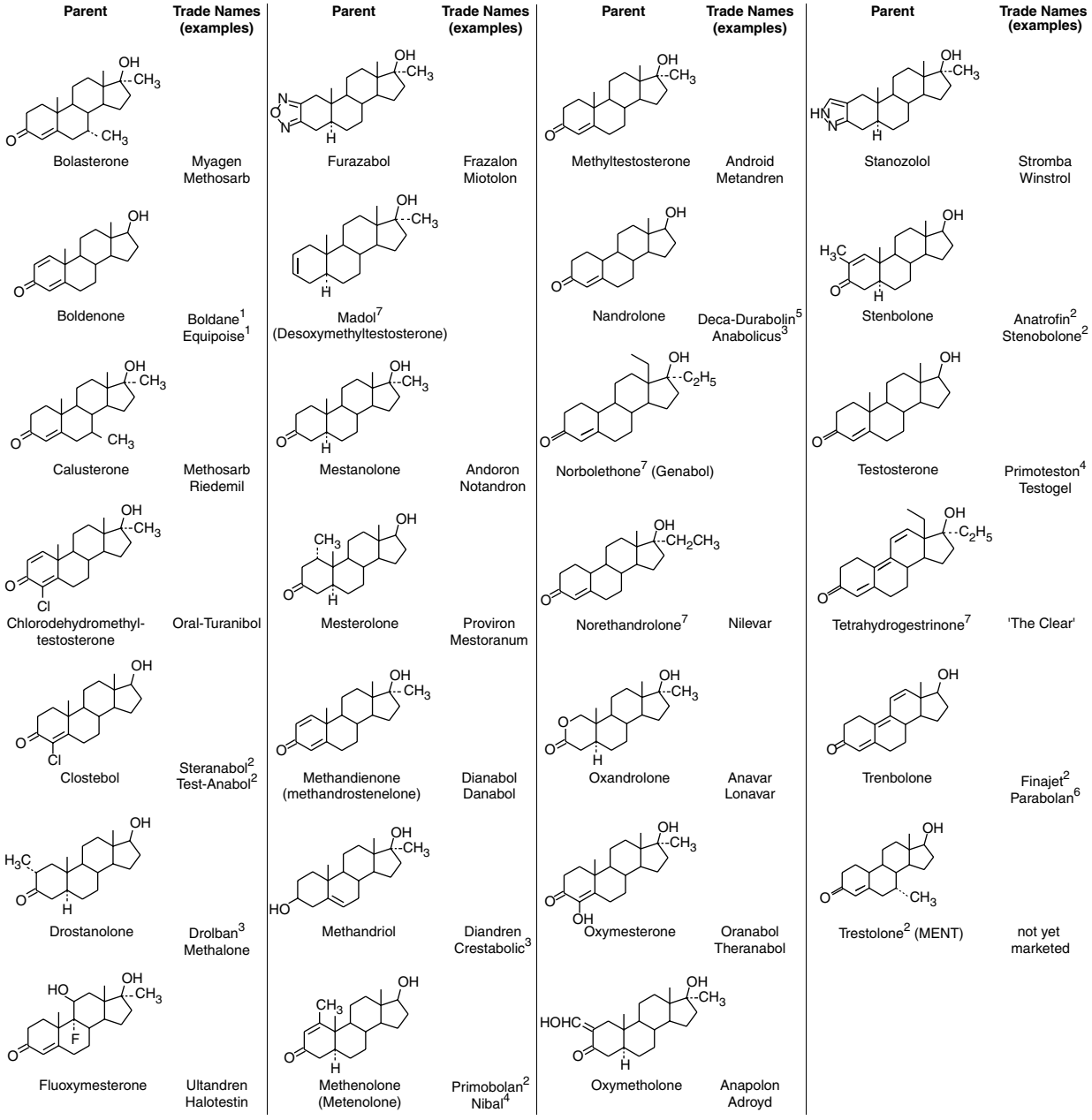


Figure 3 Structures of anabolic–androgenic steroids with corresponding diagnostic metabolites and examples of registered trade names. Superscripts ([1–6]) refer to 17β-hydroxyl-esterified preparations: [1]undecylenoate; [2]acetate; [3]propionate; [4]heptanoate; [5]decanoate; [6]hexahydrobenzylcarbonate. Superscript[7]—see the section on 'Designer steroids'.

analytical techniques such as nuclear magnetic resonance spectroscopy to confirm configuration of the structure; the interested reader is referred to the extensive review by Van Eenoo and Delbeke (2006).

With respect to prohormone supplements of testosterone, as recently reviewed by Brown *et al*. (2006), these are modelled on steroids that are endogenously produced, that is, androstenedione, androstenediol and DHEA. However, supplements of the weaker androgens DHEA or androstenedione may be of little or no benefit to healthy young men who wish to improve their strength and sporting performance if, as would be expected, any anabolic effect is primarily mitigated through peripheral conversion to testosterone. Ingestion of DHEA can result in an increase in circulating DHEA and androstenedione, but it is not resolved as to whether there is an increase in plasma testosterone, see for example Brown *et al*. (1999). This is not surprising because in the adult men the overall peripheral contribution of these precursor steroids to circulating testosterone is small. Any contribution from exogenous DHEA or androstenedione will be largely moderated by the large amount of testosterone contributed by the testis. In women, an increase in performance may be possible following ingestion of these supplements, as circulating testosterone would be expected

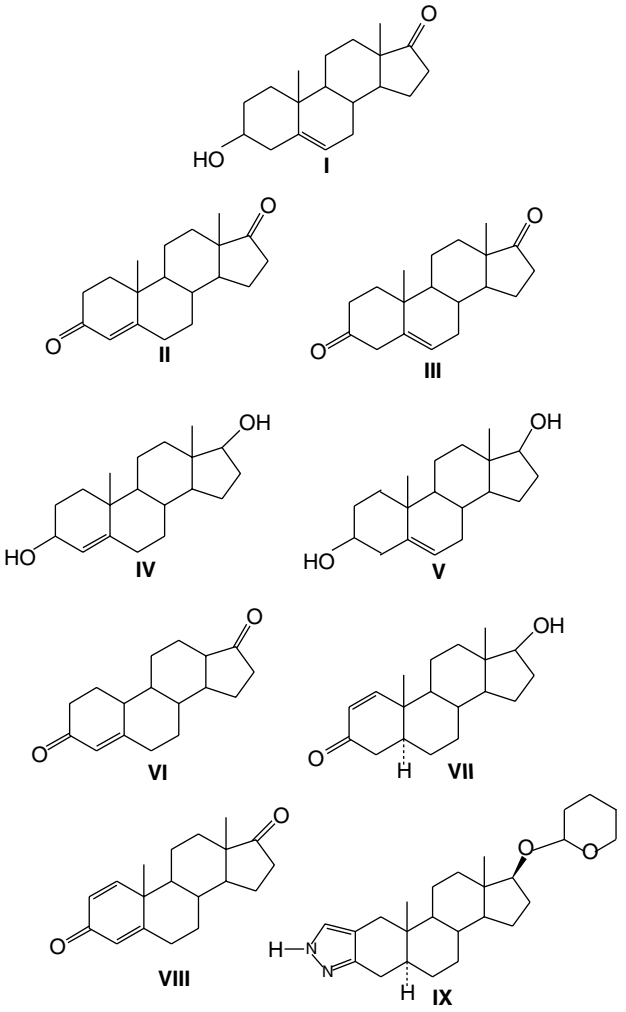


Figure 4 The 'supplements' (I) dehydroepiandrosterone (DHEA), (II) and (III) androstenedione (Δ4 and 5, versions respectively), (IV) and (V) androstenediol (Δ4 and 5 versions, respectively), (VI) 19-norandrostenedione (only Δ4 version displayed), (VII) 1-testosterone, (VIII) boldione and (IX) prostanozolol.

to increase. The plasma concentration of endogenous testosterone is approximately 1/10th that found in men and the relative proportion arising from peripheral conversion of weaker androgens is much greater. Even though only 12–14% of androstenedione is converted peripherally to testosterone (Horton and Tait, 1966; Bardin and Lipsett, 1967), this amount accounts for about half the circulating testosterone in the women. As the peripheral contribution to blood testosterone is far greater in the young adult women than the men, ingestion of modest amounts of androstenedione, DHEA or androstenediol (the natural steroid or the Δ_4 analogue) is likely to significantly raise circulating testosterone. There are modest-to-large increases in circulating testosterone following androstenedione administration to women (Leder *et al*., 2002; Kicman *et al*., 2003a; Bassindale *et al*., 2004; Brown *et al*., 2004). Women who chronically administer large doses of weaker androgens that can be converted to more potent steroids would be expected to suffer from virilizing effects. In 2004, the FDA (Food and Drug Administration), as part of its public health mission, sent warning letters to 23 companies in the United States requesting them to cease distributing androstenedione as dietary supplements (FDA, 2004).

Designer steroids

Designer anabolic steroids are considered as ones that are manufactured specifically to circumvent doping tests in human sport, and, therefore, for obvious reasons, they are supplied in a clandestine fashion. There are few examples to draw on. Classified documents (Franke and Berendonk, 1997) saved after the collapse of the German Democratic Republic revealed that, since 1983, a pharmaceutical company had produced preparations of epitestosterone propionate exclusively for the governmental doping programme. Epitestosterone, an epimer of testosterone, is a steroid with no anabolic activity but its administration with testosterone simultaneously or sequentially enables an athlete to manipulate the test for testosterone administration if the test is based solely on determination of the urinary testosterone/epitestosterone (T/E) ratio. Recently, a company in California called BALCO (Bay Area Laboratory Co-operative; Burlingame, CA, USA) attracted much media attention due to the high profile of the athletes involved, not least because of the supply of a transdermal preparation coded as 'The Cream' containing testosterone and epitestosterone, as well as a sublingual preparation of a new anabolic steroid coded as 'The Clear', which was identified from the contents of a spent syringe as tetrahydrogestrinone (THG) by the WADA-accredited laboratory within the University of California, Los Angeles (UCLA) (Catlin *et al*., 2004).

Tetrahydrogestrinone can be easily manufactured by the catalytic hydrogenation of the ethynyl group of the progestogen gestrinone (Figure 5). This relatively simple synthetic step hides the thinking that probably lay behind the design of THG. Given the close homology of their receptors, there is an overlap between the activity of progestogens and androgens, especially those xenobiotic steroids that lack the C-19 methyl group, but which activity predominates depends on whether the alkyl substituent at carbon-17 is ethynyl or ethyl. Substitution of the 17α-H with an ethynyl group on nandrolone, a 19-nor anabolic steroid with some progestational activity, will result in a potent orally active progestogen, this being called norethisterone (norethindrone), a steroid that is still used in some contraceptives today. The synthetic route is described in a seminal paper by Djerassi *et al*. (1954). However, substitution with an ethyl group on nandrolone rather than ethynyl group results in another anabolic steroid known as norethandrolone, which also has oral activity. Gestrinone, is a pharmaceutically available progestogen that lacks the C-19 angular methyl group but has a 17α-ethynyl group, and it follows that reduction of this ethynyl group to the tetrahydro product should make THG a 'potent' androgen. This is indeed the case, as subsequently THG was found to be a highly potent androgen (and progestogen) in an *in vitro* bioassay system expressing human steroid receptors (Death *et al*., 2004), and it promotes muscle accretion in orchidectomized male rats (Jasuja *et al*., 2005). Despite the presence

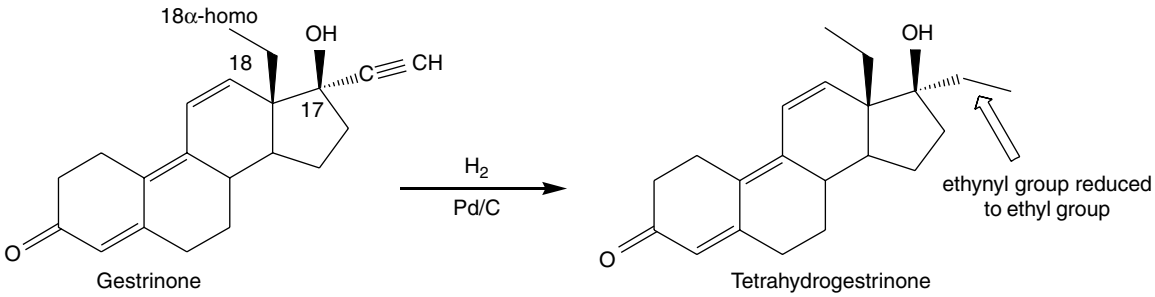


Figure 5 Catalytic hydrogenation of gestrinone to form tetrahydrogestrinone (THG). An example of a catalyst is palladium on carbon (Pd/C), as described in a procedure employed by Catlin *et al.* (2004).

of the 17α-alkyl function, which should make the steroid resistant to first-pass metabolism, it is of interest that the instructions from BALCO Laboratories were to place a few drops of the liquid preparation under the tongue, that is, a sublingual route of administration. THG was invisible on the routine gas chromatography–mass spectrometry screen employed by the WADA-accredited laboratories and necessitated the development of a liquid chromatography–mass spectrometry/mass spectrometry (LC–MS/MS) screen for its detection; for a current and detailed review on the analysis of anabolic steroids see Kicman *et al*. (2008).

Underground chemists appear also to be accessing information concerning other steroids that were synthesized several decades ago by pharmaceutical companies but were never marketed. Such steroids that have been detected until recently are norbolethone (Catlin *et al*., 2002), which was reputed to have been the active ingredient of 'The Clear' before being replaced by THG, and madol (Sekera *et al*., 2005), which is also referred to as desoxymethyltestosterone (by the WADA-accredited laboratory in Montreal, who identified this steroid around the same time as the accredited laboratory at UCLA). Although the extent of this activity appears to be limited, as screening procedures rely on targeting selecting ions for monitoring by mass spectrometry, unknown steroids may escape detection. To demonstrate how this problem may be addressed, Thevis *et al*. (2005) developed an LC–MS/MS screening method based on common fragmentation pathways and Nielen *et al*. (2006) used a combination of androgen bioassay detection and electrospray quadrupole time-of-flight mass spectometric identification.

Mechanisms of action

Anabolic steroids are thought to exert their actions by several different mechanisms. These mechanisms include modulating androgen receptor expression as a consequence of (i) intracellular metabolism and by (ii) directly affecting the topology of the androgen receptor and thus subsequent interaction with co-activators and transcriptional activity. Other mechanisms include (iii) an anticatabolic effect by interfering with glucocorticoid receptor expression; and (iv) by non-genomic, as well as by genomic pathways, in the CNS resulting in behavioural changes. These mechanisms are discussed herein.

As an adjunct, much of the physiological importance of non-genomic actions of androgens is still to be elucidated, not least with respect to androgen-induced cell-cycle progression. The induction of second messenger signal transduction cascades by steroids commonly occurs within seconds to a few minutes, in contrast to genomic activity of the classic steroid receptors that takes 30–60 min. Regarding androgens, several non-genomic mechanisms appear to be involved, including mediation by the membrane-bound sex hormone-binding globulin receptor and also a putative G-protein-coupled receptor that androgens directly bind with, as well as through stimulation of nonreceptor tyrosine kinase c-SRC. The complexity of these mechanisms is described in detail elsewhere (Cato *et al*., 2002; Heinlein and Chang, 2002a; Losel *et al*., 2003). Ultimately, gene transcription may be modulated by these 'non-genomic' pathways but a well-recognized exception is the rapid elevation of calcium ion influx by a pathway that is confined to the cytoplasm. It is not currently known whether non-genomic actions of androgens at physiological concentrations are important in skeletal muscle growth, let alone what the non-genomic effects may be evoked by the administration of anabolic steroids. For the sake of brevity, this review will only very briefly touch again on non-genomic pathways under 'Behavioural Effects' (see the section 'Behavioural mechanisms').

Intracellular metabolism and the myotrophic–androgenic index

The structural changes to testosterone by medicinal chemists were designed to enhance the protein anabolic effect relative to the androgenic effect. Unfortunately, the anabolic effects could not be divorced entirely from the androgenic effects, although some synthetic steroids present a remarkable dissociation, at least based on the myotrophic–androgenic index. It may be argued that by today's standards this *in vivo* approach, which was developed over 50 years ago, is unsophisticated given the huge developments in molecular biology since that time. Despite the criticism that this approach has attracted, it is of note that anabolic steroids with high myotrophic activity and favourable index values, for example, nandrolone (esterified), oxymetholone, methandienone and stanozolol are still available as medicines in many countries. These steroids remain desirable as a doping agent to enhance sporting performance (as evident by the statistics collated by WADA) and for

bodybuilding purposes. For this reason, it is logical to summarize this approach, based on growth of a particular skeletal muscle called the levator ani relative to that of androgenic target tissue, usually the prostate gland, and attempt to explain the underlying mechanism of dissociation of the growth of the two tissues (compared with controls).

Eisenberg and Gordan (1950) proposed the use of the rat levator ani muscle as a bioassay of protein anabolic activity; the anatomical drawings from the dissection of the male rat, displaying the location of this muscle, the prostate and seminal vesicles are displayed in this paper. The rat levator ani muscle is part of the perineal complex of striated muscles that envelope the rectum. This muscle was chosen because previous workers had reported that testosterone propionate stimulated the growth of the perineal complex in infantile rats, and, additionally, this complex was easily separated from other tissues. Eisenberg *et al*. demonstrated that the levator ani muscle in castrated, immature rats responded well to the administration of various steroids such as testosterone propionate, 17α-methyltestosterone and pituitary growth hormone (extracted from the anterior lobes of ox pituitaries). In contrast, there was a much smaller unparalleled increase in the weight of the seminal vesicles.

The foundation of the commonly used procedure of the myotrophic–androgenic index was based on a modification of the Eisenberg and Gordan method by Hershberger *et al*. (1953). Hershberger and co-workers preferred the use of the ventral part of the prostate rather than the seminal vesicles as a measure of tissue androgenic response in immature gonadectomized rats. They proposed a measure of hormonal myotrophic-to-androgenic activity using the following ratio:

Index ratio = (experimental levator ani weight − control levator ani weight)/(experimental ventral prostate weight − control ventral prostate weight) = increase in levator ani weight/increase in ventral prostate weight

Many investigators employed the approach proposed by Hershberger *et al*. (1953), but some made their own modifications to it, and others still used the seminal vesicles as a bioassay of androgenicity.

Like the index value, the myotrophic or androgenic effects were themselves expressed as ratios to other reference steroids, for example, 17α-methyl testosterone or testosterone for oral routes and testosterone propionate for parenteral routes. A comprehensive comparison of the anabolic and androgenic activities of many anabolic steroids and their dissociation index is given elsewhere (Potts *et al*., 1976) but some examples are displayed in Table 1.

Kruskemper (1968) discusses the many failings of the procedures used for determining the myotrophic–androgenic index, for example, the seminal vesicles react more slowly to certain androgens, so that with short test administration, distortions can arise in favour of the myotrophic effect. The harshest criticism of this index was given by Nimni and Geiger (1957), Scow and Hagan (1957) and Hayes (1965). Testosterone administration for 56 days to young gonadectomized rats (castrated at 20–23 days of age) had no effect on the growth of the thigh muscle compared with controls, yet there was considerable growth in the perineal musculature (Scow, 1952; Scow and Hagan, 1957). Testosterone propionate or norethandrolone (17α-ethyl-19-nortestosterone; also an anabolic steroid) administration promoted the growth of the levator ani muscle even in young normal or castrated rats on a protein-free diet, that is, a local anabolic effect proceeding at the expense of catabolic processes in other organs. Hayes (1965) stated that the rat levator ani muscle is not homologous to this muscle in other species, that is, it is not a typical sphincter muscle and does not lift the anus in rodents but is part of the male reproductive system. Thus, Hayes renamed the levator ani muscle, calling it the dorsal bulbocavernosus. All three groups of workers showed that the levator ani muscle reflects a general genitomyotrophic response rather than an overall response to androgens. Later, Hervey (1982) claimed that the male rat's characteristics are determined shortly after birth (due to a brief secretion of testosterone), and, thereafter, any increase in body mass is not affected by androgens.

Table 1 Comparison of myotrophic and androgenic activities of anabolic steroids—examples were drawn from a much more comprehensive table (with referenced papers) presented by Potts *et al*. (1976)

Steroid	*Route*	*Reference steroid*	*Activity*		*Index value*
			Myotrophic	*Androgenic*	
Chloromethyl T	p.o.	17α-MeT	0.5	0.10–0.15	3–5
Methandienone	p.o.	17α-MeT	0.60	0.20	3
Methenolone acetate	p.o.	17α-MeT	0.86	0.12	7
Nandrolone decanoate	par.	T propionate	3.29–4.92	0.41–0.31	12.1–10.6
Norbolethone[a]	par.	T propionate	3.44	0.15–0.17	20
Norethandrolone	par.	T propionate	0.77–1.0	0.06–0.38	2–16
Oxandrolone[b]	par.	17α-MeT	3.22	0.24	13
Oxymesterone	p.o.	17α-MeT	1.34	0.42–0.61	2.2–3.2
Oxymetholone	p.o.	17α-MeT	3.20	0.45	7.1
Stanozolol	p.o.	17α-MeT	2.0–3.7	0.33–0.52	6–10.6
T	p.o.	17α-MeT	0.36	0.28–0.50	0.7–1.3

Abbreviations: par., parenteral; T, testosterone.
[a]Norbolethone was developed in 1966 but it was never commercially marketed. It was detected in two urine samples from an athlete (August 2001, March 2002) by Catlin *et al*. (2002). A US Government Investigation revealed that a chemist based in Champaign (IL, USA) synthesized this steroid and it was distributed by a company called BALCO Laboratories (see also the section 'Designer steroids').
[b]Only the activity for the parenteral route available.

Contrary to the opinions described above, there is nonetheless biochemical evidence that suggests that the genito-myotrophic response of the levator ani muscle may serve as an indicator of the general myotrophic responses in the developing rat for the following reasons. The same classic androgen receptor can be characterized in the prostate, the bulbocavernosus/levator ani muscle and typical skeletal muscles of the rat (Krieg and Voigt, 1977). Nandrolone (19-nortestosterone) and 5α-DHT have a higher binding affinity than testosterone with the receptor. The prostate has 7 times the concentration of androgen receptors than the bulbocavernosus/levator ani muscles which in turn has 10 times more than other skeletal muscle. *In vitro* studies by Gloyna and Wilson (1969) and Massa and Martini (1974) have shown that 5α-reductase activity is very high in rat sexual tissue such as the prostate and seminal vesicles but negligible, if at all, in skeletal muscle such as the levator ani and thigh muscle. Intracellular DHT is, therefore, low in skeletal muscle, and it is worth emphasizing that its presence is further diminished because of the high activity of the enzyme 3α-hydroxysteroid-dehydrogenase in this tissue (and cardiac tissue as well), the enzyme that converts DHT irreversibly to 3α-androstanediol (Massa and Martini, 1974; Smith *et al*., 1980). The very low activity of 5α-reductase in skeletal (and cardiac) muscle was subsequently confirmed by other investigators (Krieg *et al*., 1976; Bartsch *et al*., 1980), and although the enzymatic activity within the levator ani appears to be significantly higher, it still represents only 5% of that within the prostate. The rat levator ani may be a somewhat atypical striated muscle because of its greater concentration of androgen receptors, but, due to its very low 5α-reductase activity, it can also be argued that it is not a typical part of target tissues associated with the reproductive system. Celotti and Cesi (1992), in their review of possible mechanisms of action of anabolic steroids, discuss that the peculiar androgen sensitivity of this muscle is intermediate between that present in the skeletal muscles and that of the prostate. The myotrophic effect of anabolic steroids may be reflected by the amplified response of the levator ani muscle due to its higher concentration of androgen receptors, an effect that is not apparently sufficient in other (typical) rat skeletal muscles to be observed using differences in weight (compared with controls) as the measurand.

A possible basis for increasing the myotrophic-to-androgenic ratio may be by exploiting the fundamental difference between the 5α-reductase concentrations in skeletal muscle and androgenic tissue. One way of increasing the anabolic–androgenic dissociation is to administer a steroid that has a greater binding affinity for the androgen receptor but upon reduction to a 5α-metabolite has a lesser affinity. Among the anabolic steroids, 19-nortestosterone (nandrolone) was one of the first synthesized, the most used and probably the best studied. Although DHT has a greater binding affinity for the androgen receptor than its parent steroid testosterone, by contrast the 5α-reduced form of 19-nortestosterone, 5α-dihydro-19-nortestosterone, has a lesser binding affinity than its parent steroid 19-nortestosterone (Toth and Zakar, 1982). Hence, in androgenic tissue, testosterone is converted to a more potent metabolite, whereas 19-nortestosterone is converted to a less potent one. As 5α-reduction occurs readily in androgenic tissue but is negligible in skeletal muscle, this explains why 19-nortestosterone has a greater myotrophic-to-androgenic ratio when compared with testosterone (Figure 6). If the model is correct, such a diminishment in androgenic activity should not be confined to the accessory reproductive tissues in the human such as the prostate, but also in non-genital target tissues where clear roles for the metabolism to DHT have been defined such as the male patterns of facial and body hair growth, thus allowing more muscle per whisker. Moreover, even where testosterone rather than DHT appears to stimulate other secondary sexual characteristics, such as voice deepening, with the discovery of two isozymes of 5α-reductase (for review see Russell and Wilson, 1994), it cannot be ruled out that some of these actions attributed to testosterone need to be re-evaluated, the results of which may have relevance to the applicability of anabolic steroids with a high myotrophic–androgenic index. Much of the knowledge of the separate roles of testosterone and DHT came from 5α-reductase deficiency syndrome, but these effects are all ascribed to mutations in the type 2 isoenzyme (Randall, 1994) and the biological role of the 5α-reductase type 1 is harder to ascertain as there is no recognized type 1 deficiency. For example, type 2 5α-reductase appears not to be necessary for the sebaceous gland response to androgens and the development of acne, but it is now known that the principal isoenzyme in this gland is the type 1 form (Thiboutot *et al*., 1995; Sato *et al*., 1998). As an adjunct, dutasteride (Avodart; manufactured by GlaxoSmithKline), which inhibits both type 1 and type 2 5α-reductases and is used in the treatment of benign prostatic hyperplasia (Clark *et al*., 2004) and male pattern hair loss (Olsen *et al*., 2006), appears not to be helpful in the treatment for acne vulgaris (Leyden *et al*., 2004). This suggests that further work at the molecular level is required to better understand the action of androgens on sebaceous gland function.

This mechanism of myotrophic–androgenic dissociation does not explain why other anabolic steroids that do not undergo 5α-reduction, for example, those with an extra double bond in the A-ring, such as chlorodehydromethyltestosterone and methandienone (Schanzer, 1996), have a favourable mytotrophic–androgenic index. Even so, it is possible that that myotrophic–androgenic dissociation may occur, simply because the effect of the particular steroid cannot be amplified by 5α-reduction in androgenic target tissues, in common with the hypothesis proposed for the differential action of a steroidal SARM (see the section 'Selective androgen receptor modulators' for an explanation of the term) called MENT (7α-methyl-19-nortestosterone; trestolone) (Agarwal and Monder, 1988; Kumar *et al*., 1992), as reviewed by Sundaram and Kumar (2000).

Recently, as part of investigations to assess whether the designer steroid THG had anabolic and androgenic properties (see also next section), three papers report the effects of its administration on the growth of the levator ani, prostate and seminal vesicles compared with control steroids (Jasuja *et al*., 2005; Labrie *et al*., 2005; Friedel *et al*., 2006a). Notwithstanding the possible differences in pharmacokinetics and bioavailability between THG and the control steroids administered, there appeared to be little myotrophic–androgenic

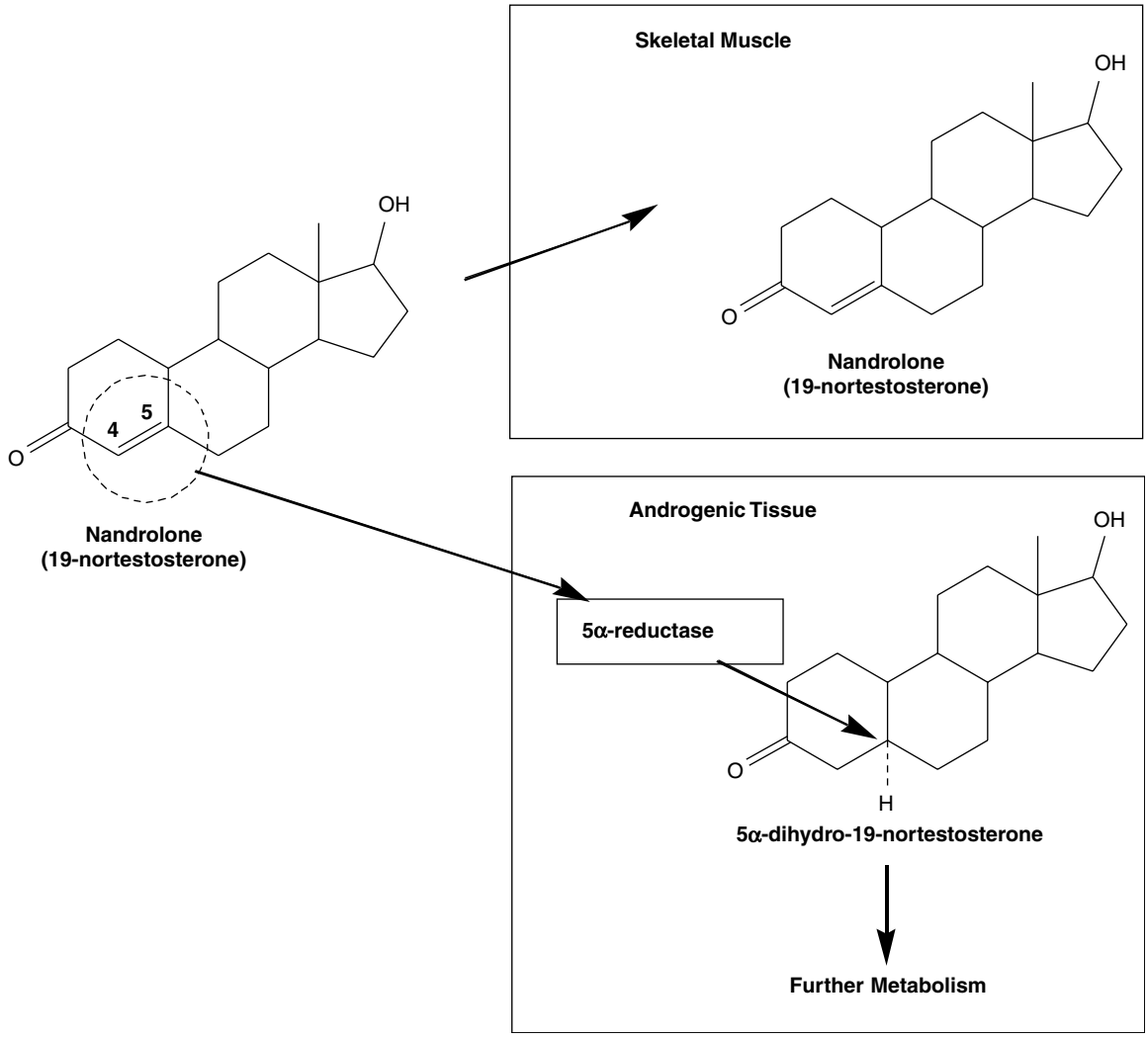


Figure 6 In androgenic tissues, nandrolone (19-nortestosterone) is readily converted by the enzyme 5α-reductase into 5α-dihydro-19-nortestosterone, i.e., the double bond between C4 and C5 is reduced. This metabolite binds with weaker affinity to the androgen receptor compared with the parent steroid. Further metabolism can occur because of the high activity of the enzyme 3α-hydroxysteroid-dehydrogenase (which reduces the 3-oxo group) in androgenic tissue. In skeletal muscle, 5α-reductase activity is negligible and, therefore, the parent steroid itself binds with strong affinity to the androgen receptor. It follows that there is a favourable disassociation of the myotrophic effects from the androgenic effects of nandrolone and also that there is a greater myotrophic-to-androgenic ratio when compared with testosterone.

dissociation, but, nonetheless, the bioassays clearly demonstrated that THG had anabolic and androgenic activity *in vivo*, and, therefore, belonged within the banned doping class of anabolic agents in sport, as defined by WADA.

As a final and very important point, it is of note that complete dissociation has not been achieved with any anabolic steroid synthesized, and, therefore, the chronic administration of these drugs, even those with a very high myotrophic–androgenic index value, such as found with nandrolone (19-nortestosterone), will result in hirsutism and, eventually, virilization of women and children.

Androgen receptor expression and the importance of coregulators

The androgen receptor belongs to the family of nuclear receptor superfamily (Mangelsdorf *et al.*, 1995), these intracellular receptors eliciting so-called 'classical' or genomic, actions by interacting with DNA and modulating transcription. A DNA-binding domain, a ligand-binding domain and at least two transcriptional activation domains, characterize these receptors. Apart from binding with the steroid, the ligand-binding domain also functions in dimer formation and mediates transcriptional activation. The DNA-binding domain targets the receptor to specific DNA sequences known as steroid (or hormone) response elements. On the receptor, the DNA-binding domain consists of two subdomains called 'zinc-fingers'; each subdomain contains four cysteine residues that coordinate with a zinc atom, thus, stabilizing the 'finger' structure. The zinc-fingers are inserted between specific grooves of the DNA helix, thus, allowing maintenance of DNA-binding activity.

A general model of steroid receptor action is displayed in Figure 7. In the absence of hormone, it is by and large accepted that steroid receptors exist as an inactive oligomeric complex, being sequestered by the heat-shock protein (Hsp), Hsp90, which acts as a molecular chaperone. Hsps are

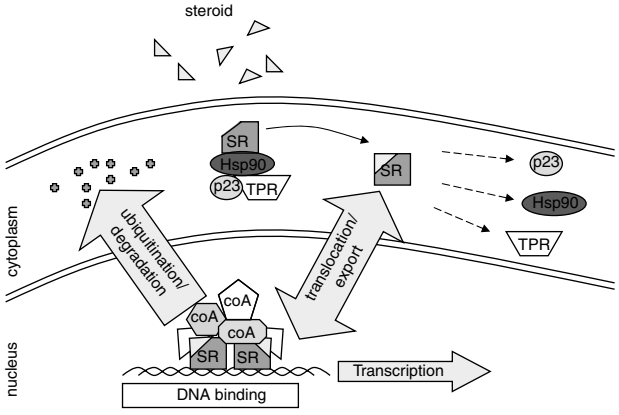


Figure 7 In the absence of hormone, the steroid receptor exists as an inactive oligomeric complex with the molecular chaperone heat-shock protein, Hsp90, and p23, and co-chaperones utilizing tetratricopeptide repeat (TPR) motifs. After hormone binding, the receptor–Hsp90 complex disassociates and the activated receptor is translocated into the nucleus. Activated receptors interact as homodimers with the steroid response element on the chromatin, triggering the formation of a transcription complex, a cluster of coregulators resulting in gene activation, transcription of the gene, protein translation, and a resultant alteration in cell function, growth or differentiation. This figure is redrawn in the own author's style but was based on part of the figure in the article by Weigel and Moore (2007).

so-called because they were discovered to accumulate under stress conditions including within heat-traumatized cells, but many are present and functionally important under normal conditions; they are named according to their molecular weight in kilodaltons. Another chaperone called p23 stabilizes the aporeceptor complex by blocking Hsp90 in the ATP-bound substrate conformation. Co-chaperones utilizing tetratricopeptide repeat motifs are necessary for docking of the Hsp90. As an adjunct, other chaperones, called Hsp40 and Hsp70 and an organizing protein called Hop (heat-shock organizing protein) are important in the assembly of the steroid receptor–Hsp90 complex. Picard (2006) gives a clear overview of molecular chaperones and cofactors that are relevant to steroid receptor action. Phosphorylation of the receptors is also important in regulation of receptor function (Weigel and Moore, 2007).

The steroid receptor–Hsp90 complex appears to be necessary for the receptor to stabilize in a conformation for binding to the ligand with high affinity and also to maintain its solubility in the cell. It is generally accepted that, although the receptor is held in this complex, it is inactive as a transcription factor, that is, the Hsp90 complex acts as a repressor of transcriptional activity by preventing one or several of the following: nuclear localization, dimerization, DNA binding and interaction with transcriptional co-activators.

Steroids are relatively small molecules, for example, testosterone has a molecular weight of 288, and they can passively diffuse into cells. In target tissues, that is, the cells that contain steroid receptors, the hormone binds to the receptor ligand-binding domain, causing dissociation of the receptor–Hsp90 complex, the resultant conformational (allosteric) change making the receptor active. In the case of the androgen (and glucocorticoid) receptor, the chaperone complex resides in the cytoplasm, and following dissociation from the chaperone the activated receptor is translocated into the nucleus. Activated receptors interact as homodimers with the steroid response element on the chromatin, the effect of two receptors binding being cooperative (greater affinity and stability). This attachment to the DNA, in turn, triggers the formation of a transcription complex, a cluster of coregulators (also called comodulators) that fit around the receptors like 'pieces in a jigsaw puzzle'. Coregulators can be either positive or negative regulatory proteins, referred to as co-activators or corepressors, respectively (Perissi and Rosenfeld, 2005). Co-activator and corepressor complexes are required for nuclear receptor-mediated transcriptional regulation, generally liganded receptors recruiting co-activators resulting in gene activation, transcription of the gene, translation and a resultant alteration in cell function, growth or differentiation.

The function of the transcriptional activation domains on the receptor is to mediate the binding of the receptor to the comodulators. The receptor has an N-terminal activation function-1 (AF-1) and a second activation function-2 (AF-2) in the C-terminal ligand-binding domain. The mechanisms of AF-1 and AF-2 gene activation, with emphasis on AF-1 and AF-2 conformation and co-activator binding, have been reviewed by Warnmark *et al*. (2003). The mechanism of AF-1 gene activation is not well understood due to the lack of conformational information but, by contrast, many crystal structures of the ligand-binding domain of different nuclear receptors have been achieved, allowing a fuller understanding of AF-2-mediated transcriptional activation. AF-2 is dependent on ligand binding to the receptor for its activity, which causes the folding of a C-terminal helix (helix-12), acting as a lid over the ligand pocket upon ligand binding. This folding creates the activation surface/AF-2 domain, allowing the docking of AF-2 co-activators and the formation of a charge clamp that stabilizes co-activator interaction, these co-activators having the leucine-X-X-leucine-leucine (LXXLL) motif necessary for such interaction (X is any amino acid).

The molecular biology of the androgen receptor has been reviewed by Klocker *et al*. (2004). In contrast to other steroid receptors, most of its transcriptional activity is mediated through the N-terminal AF-1 domain, there being a reduced capacity of AF-2 in the androgen receptor to recruit LXXLL-containing co-activators. Instead, it has been suggested that the AF-2 of the androgen receptor acts primarily as an interaction platform for the recruitment of co-activators to the N-terminal region, this regulation of gene expression through the intradomain interaction and communication being unique to this receptor.

To date, several families of co-activator proteins have been identified but only two direct inhibitors of androgen receptor function have been identified *in vivo*, SHP and DAX-1, these being atypical orphan receptors that lack DNA-binding domains. Using X-ray crystallography, the interaction between peptide segments of SHP containing LXXLL-like motifs and the ligand-binding domain on the androgen receptor was investigated, and it was found that the LKKIL motif formed a complex, binding with a hydrophobic groove on the androgen receptor (Jouravel *et al*., 2007). It was

suggested that this transcriptional activity of androgen receptors might be inhibited by SHP competing for binding to androgen receptor co-activators. The binding motif to the androgen receptor by DAX-1 is still to be elucidated. Another corepressor, FoxG1, appears to be a likely candidate for interaction with the androgen receptor *in vivo* but studies are necessary to prove whether this is the case (Obendorf *et al*., 2007).

Anabolic steroids bind to the androgen receptor with different affinities. Saartok *et al*. (1984) compared the relative binding affinity of the anabolic steroids ethylestrenol, fluoxymesterone, mesterolone, methandienone, methenolone, 17α-methyltestosterone, nandrolone and oxymetholone to that of tritiated methyltrienolone (17β-hydroxy-17α-methyl-4,9,11-estratrien-3-one; a steroid resembling trenbolone but with a 17α-alkyl substituent) to androgen receptors in cytosol isolated from skeletal muscle and the prostate gland of the rat and skeletal muscle of the rabbit. The order of relative binding affinities in comparison with methyltrienolone, which had the strongest affinity, was nandrolone > methenolone > testosterone > mesterolone; a group which had relatively high and generally similar affinity for the androgen receptor in all three tissues. These investigators did not rank 17α-methyltestosterone, but it had a relative binding affinity of 0.1, which made it an efficient competitor. The relative binding with fluoxymesterone, methandienone and stanozolol was much weaker and that with oxymetholone and ethylestrenol was too low to be determined. There is a large discrepancy as to what is known about the *in vivo* activities of these steroids compared with their *in vitro* activity, even taking into account possible differences in the bioavailability and clearance of these steroids (not least determined by the affinity to sex hormone-binding globulin in the blood circulation). For example, oxymetholone and stanozolol have low relative binding affinity compared with 17α-methyltestosterone in the *in vitro* study, but, conversely, these steroids have a relatively high myotrophic activity compared with the same steroid when administered to the castrated rat (see Table 1). Furthermore, Feldkoren and Andersson (2005) found that stanozolol and methandienone have significantly lower binding affinities compared with testosterone but all three steroids were potent activators in a cell-based androgen receptor-dependent transactivation assay. Clearly, the degree of physical binding to the androgen receptor, as measured by ligand-binding assays, does not fully explain the biological activity of anabolic steroids. Distinct target gene expression profiles due to androgen receptor activation by structurally different androgens has also been reported (Holterhus *et al*., 2002), the study including three anabolic steroids nandrolone, oxandrolone and stanozolol, together with what the investigators term three 'virilizing androgens' (testosterone, DHT and methyltrienolone) and two testosterone precursors (DHEA and androstenedione). The model used was three structurally different androgen promoter constructs in co-transfected Chinese hamster ovary cells. All the steroids proved to be potent activators of the androgen receptor, but the anabolic steroids and the testosterone precursors showed characteristic promoter activation profiles distinct from the virilizing androgens. The assumption is that the specific ligand-induced conformation determines how the hormone receptor complex can specifically interact with coregulators and neighbouring transcription factors and also that the transactivation capability depends on the structure of the response element. Even though many co-activators have been identified as enhancing the ligand-induced transcriptional activity of the androgen receptor, their relative importance with respect to particular cell types and tissues is unclear (Heinlein and Chang, 2002b). The emerging knowledge concerning androgen receptor interaction with its coregulators in different tissues clearly has relevance to understanding how anabolic steroids exert their actions and will give further insight into how favourable anabolic–androgenic dissociation may be achieved.

Most recently, *in vitro* bioassays have been employed to determine that the designer anabolic steroid THG is indeed a potent androgen. Death *et al*. (2004) demonstrated that THG was about one order of magnitude more potent than nandrolone, testosterone and trenbolone in yeast cells expressing human androgen receptors. Friedel *et al*. (2006b) also used a reporter gene assay based in a yeast strain containing transfected androgen receptor constructs and found that THG was about 10 times lower than the EC_{50} of the reference substance DHT. (Jasuja *et al*. (2005) found that THG upregulated androgen receptor expression in mesenchymal multipotent cells by measuring the translocation of the receptor to the nucleus using immunohistochemical and analyses, but this was not significantly different from DHT. The authors make the important point that it is not known whether yeast-based systems express the repertoire of coregulators that is present in mammalian androgen-responsive tissues. Labrie *et al*. (2005) studied the genomic signature of THG and compared it with the effects of DHT on gene expression in mouse tissues by extracting RNA, converting it to cDNA and then transcribing it *in vitro* to produce biotinylated cRNA for analysis. These investigators found that THG and DHT modulated in a similar fashion 671 genes in the mouse levator ani muscle, 95 genes in the gastrocnemius muscle and 939 genes in the prostate.

The use of *in vitro* assays based on androgen receptor expression, as described above, can help to assess whether future designer steroids have anabolic–androgenic activity, and can help to minimize *in vivo* experiments. These approaches can provide useful evidence to government agencies involved in the regulation of drugs to protect public health. Moreover, the employment of such assays should be of particular benefit to sporting authorities to help stifle legal challenges based on the premise that new designer steroids have unproven anabolic activity and thus should not be subject to doping control and the penalties associated with their administration.

Anticatabolic activity

It is accepted that the administration of anabolic steroids to healthy women and children has an anabolic effect, and that with the virilizing effects, there is a gain in muscle mass and strength. However, for many years, it was difficult to prove conclusively that the administration of these steroids had a myotrophic effect in healthy young sportsmen, as discussed

by Ryan (1976) (see the section 'Anabolic steroids as performance enhancers in sport'). Around that period, an interesting but speculative biochemical explanation for this difference in response between the sexes was that due to the exposure to testosterone during puberty in men, there is a downregulation of receptors (decrease in responsiveness of receptors often followed by decrease in numbers) in the skeletal muscle and that the androgen receptor population is then saturated with testosterone in the adult, so that no further response can be induced by pharmacological doses of androgens (Wilson, 1988). It was therefore reasoned that any possible myotrophic effect from administration of anabolic steroids to eugonadal men could be via an anticatabolic mechanism rather than a direct anabolic effect. However, the proposed downregulation of androgen receptors in skeletal muscle because of increased androgen exposure was based on a few animal studies at that time (Dahlberg *et al*., 1981; Rance and Max, 1984) and conflicting evidence was presented by Michel and Baulieu (1980) and more recently by others, for example, Antonio *et al*. (1999). Indeed, Antonio *et al*. speculate that upregulation may occur with the administration of pharmacological amounts of androgens, converting muscles that normally have a minor, or no response, to muscles with enhanced androgen responsiveness. Androgen receptor regulation in different groups of skeletal muscle in response to physiological and supraphysiological exposure to testosterone is intricate, let alone what may occur following administration of xenobiotic anabolic steroids, and the interested reader is referred to the detailed review by Dr F Kadi in the same issue of this journal.

Indirect evidence of an antiglucocorticoid effect comes from a case report concerning partial androgen insensitivity syndrome (Tincello *et al*., 1997). A patient with a single amino-acid mutation in the androgen receptor DNA-binding domain (Arg-608 to Lys), which explained his lack of overall response to high-dose androgen treatment at different times in his life, nonetheless, could be induced into a positive nitrogen balance with testosterone administration. An appealing explanation for this finding is that anabolic steroids act as glucocorticoid receptor antagonists. Most binding studies, however, indicate that anabolic steroids have very low binding affinity for the glucocorticoid receptor (Hickson *et al*., 1990), a notable exception being THG, which binds with high affinity (Friedel *et al*., 2006b). An alternative hypothesis, therefore, is that anabolic steroids may interfere with glucocorticoid receptor expression at the gene level.

Over the years, it has become apparent that the endocrinology of skeletal muscle is highly complex, and there is a delicate balance between synthesis and breakdown during growth, health, disease and ageing, as considered by Sheffield-Moore and Urban (2004). It is this complexity that makes it challenging to resolve the significance of anabolic steroids as anticatabolic (and anabolic) agents across the spectrum, from the healthy athlete who desires faster recovery from arduous training schedules where cortisol may be somewhat raised (Hervey, 1982) to the patient with severe physical trauma, such as from a burn injury, where there is extreme hypercortisolaemia and hypoandrogenaemia (Sheffield-Moore and Urban, 2004).

Behavioural mechanisms

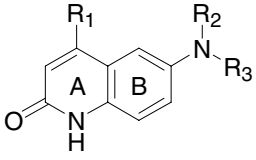
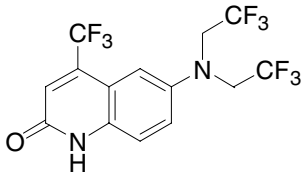
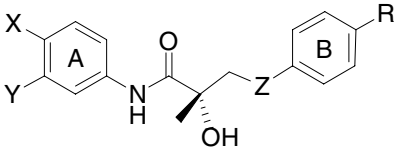
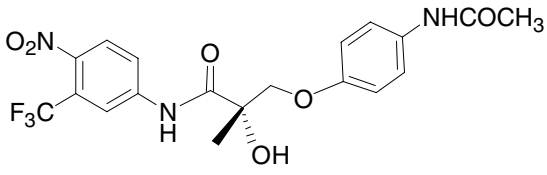
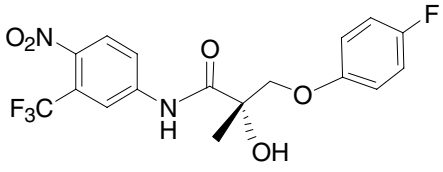
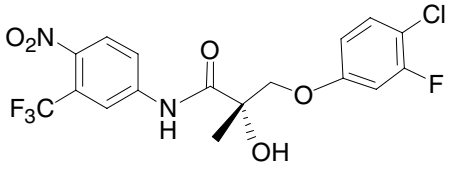
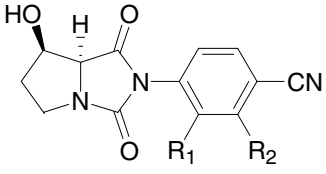
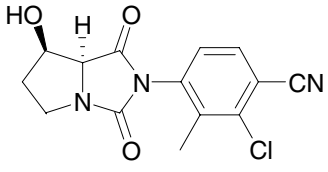
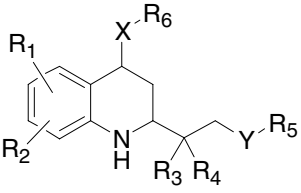
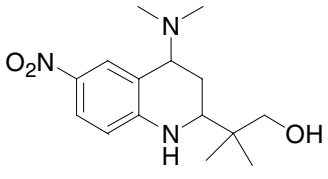
The behavioural effects of androgens/anabolic steroids in men and women, including those concerning sexual behaviour, cognitive abilities, aggression and mood, have been reviewed by Lukas (1996), Christiansen (2001, 2004) and Kuhn (2002) and are also discussed in the National Institute on Drug Abuse (NIDA) Research Monographs (Katz and Pope, 1990; Svare, 1990; Yesalis *et al*., 1990). Androgens are critical to the human male sexual behaviour and they can also enhance female sexual desire and arousal. Testosterone appears to play an important role in cognitive functioning, such as attention and alertness, memory and spatial skills, although based on the conclusions of a limited number of studies. With respect to mood, there are significantly positive correlations of endogenous androgen concentrations with a sense of well-being and joyfulness, and negative correlations with depression and anxiety. Major mood syndromes can arise with anabolic steroid use, including mania or hypomania (mania of a mild type) during exposure and depressive symptoms during steroid withdrawal (Pope and Katz, 1994). Anabolic steroid administration is also associated with increased aggression, especially in high-dose users, but this is not a foregone certainty given that the interaction between androgens and behaviour in men and women is complex. It is an entirely reasonable hypothesis that the athlete may learn to recognize and harness the increase in aggression that can arise with steroid use to help drive their training and increase their competitiveness (Brooks, 1978). Furthermore, male athletes who administer anabolic steroids and then withdraw just before competition in anticipation of a drug test may then experience (in the author's opinion) a lack of motivation and possibly depression, because they will be in a state of androgen deficiency, taking time for testicular steroidogenesis to recover. In an effort to avoid this problem, it is possible that some athletes may switch to using fairly small doses of short half-life formulations of testosterone for replacement purposes in the hope that, at the time of collection of their sample for drug testing, the urinary testosterone/epitestosterone ratio will be below the WADA reporting threshold of 4.

Clark and Henderson (2003) have summarized the literature with respect to the effects of anabolic steroids on the neural circuits that underlie behavioural effects; their review focusing on animal models and steroid exposure that mimic human abuse regimes. Androgen receptors mediate the effects of anabolic steroids in the mammalian brain; the expression of progestogen and oestrogen receptors may also be affected. Non-genomic pathways are important too, the best-characterized example being the allosteric modulation of $GABA_A$ receptor function by anabolic steroids, possibly through a putative binding site for anabolic steroids residing within the transmembrain domain of the receptor. Induction of aggression by anabolic steroids appears to overlap with neural circuits underlying the regulation of aggression by endogenous androgens, these being systems utilizing GABA, serotonin and arginine vasopressin.

Selective androgen receptor modulators

Many anabolic steroids were synthesized and their biological activity characterized (myotrophic–androgenic index, metabolic studies in animal and man) over 40 years ago, at a time when molecular endocrinology was in its infancy. With the knowledge gained in the 1980s and 1990s as to how selective oestrogen receptor modulators, such as tamoxifen and raloxifene, may work at molecular level (Jordan, 1998), perhaps it is not surprising that there is currently an interest in the possibility of modulating the androgen receptor in a similar manner. The development of SARMs, including their ligand interactions with the androgen receptor, is reviewed by Bhasin *et al*. (2006). Tissue selectivity may be achieved by synthesizing ligands that modulate the expression of the androgen receptor by inducing specific conformational changes that affect its interaction with coregulators. Androgens, steroidal or nonsteroidal, that offer tissue selectivity based on a divergence in intracellular metabolism are also included under the term SARM, such as the steroid MENT, which cannot undergo 5α-reduction (Kumar *et al*., 1992) but is almost certainly aromatized to an active oestrogen (Lamorte *et al*., 1994; de Gooyer *et al*., 2003). Indeed, the role of 5α-reductase appears to play a critical part in

Table 2 Non-steroidal AR agonists (Gao and Dalton, 2007a, b)

Chemotype	General chemical structure	Lead compound
Quinolinone analogues		LGD2226 
Aryl propionamide analogues		S4 
		S1 
		C6 
Hydantoin analogues		BMS564929 
Tetrahydro-quinoline analogues		S40503 

Abbreviation: AR, androgen receptor.
Table reproduced from the article in *Drug Discovery Today* by Gao, W and Dalton, JT (2007) *Drug Discov Today* **12**:241–248, with kind permission from Elsevier (2008).

determining the tissue-specific expression of SARMs (Gao and Dalton, 2007a). Notwithstanding, the clinical applications of steroidal androgens are generally limited by poor tissue selectivity, pharmacokinetics and toxicity, and it is hoped that the amenability to structural modifications of nonsteroidal ligands will overcome these limitations. The current nonsteroidal SARM pharmacophores are analogues of aryl propionamide, bicyclic hydantoin, quinoline and tetrahydroquinoline (Gao and Dalton, 2007b) (Table 2).

Clinically, SARMs may offer unique therapeutic potential to androgen therapy (Negro-Vilar, 1999; Roy *et al*., 2001; Wolf and Obendorf, 2004) and ultimately those that maintain the anabolic actions of androgens without causing virilization would greatly expand the therapeutic options for women (Gao and Dalton, 2007b). Negro-Vilar (1999) gives a wish list of the desired profile of activity of SARMs, these being tailored to a number of male and female applications. Generally, all include an anabolic effect in muscle and bone, but the androgenic effects are modified to varying degrees from stimulatory, to weak or neutral, depending on the disease state. For example, for the treatment of hypogonadism in elderly men, it is important to minimize induction of growth of the prostate gland to avoid increasing the risk of developing benign prostatic hypertrophy or cancer of the prostate, and, thus, an SARM could be administered with weaker to no activity in this gland. SARMs also could be useful, but not merely confined to, the treatment of osteopaenia, osteoporosis and sarcopaenia in elderly men and postmenopausal women (assuming sufficient anabolic–androgenic dissociation can be achieved for the latter), glucocorticoid-induced osteoporis, HIV wasting, cancer cachexia and different types of muscular dystrophies.

Clinical applications

The clinical applications of anabolic steroids has been reviewed recently by Basaria *et al*. (2001) and Shahidi (2001). Historically, the usefulness of anabolic steroids in reversing the catabolic state of patients had not proved convincing and, by the end of the 1980s, many anabolic steroids had been withdrawn as licensed products and those remaining were limited for the purpose of hormone replacement therapy and the treatment of specific diseases (see next paragraph). A detailed analysis of the plethora of clinical reports, including uncontrolled trials and case studies, together with consideration of the risks versus benefits of various anabolic steroids for protein-building purposes is beyond this review. What is especially of note, however, is that lately the potential of anabolic steroids as therapeutic agents to increase weight, lean body mass and strength is being currently revisited. Anabolic steroids, such as testosterone esters, and the 17α-alkylated steroids oxymetholone and oxandrolone, may play a significant role in the treatment of cachexia associated with AIDS, severe burns and renal failure, where nutrition and standard care have been ineffective, as reviewed by Basaria *et al*. (2001). Further, nandrolone decanoate has been demonstrated to be effective in countering sarcopaenia in patients receiving dialysis (Johansen *et al*., 1999, 2006) and trestolone (MENT) could be a promising new androgen therapy for sarcopaenia (loss of muscle and strength in senescence). In the older woman, oxandrolone administration stimulates muscle protein anabolism (Sheffield-Moore *et al*., 2006), but the role of anabolic steroid therapy in women with wasting syndromes very much needs to be evaluated (Basaria *et al*., 2001). Notwithstanding the above, a number of regulatory and conceptual issues are hindering progress in deciding which clinical conditions may benefit from intervention with anabolic steroids (Bhasin *et al*., 2006), not least what outcomes should constitute evidence of efficacy in clinical trials. For example, although, theoretically, an increase in lean body mass and weight in HIV-infected individuals suffering from weight loss should lead to improved physical functioning and quality of life, and ultimately to increased survival, this has not been demonstrated (Johns *et al*., 2005). Carefully designed randomized trials may eventually give the definitive answers as to the clinical usefulness of therapy with anabolic steroids, and whether xenobiotic anabolic steroids offer any advantage over supraphysiological doses of testosterone to men. In designing trials involving women, to help reduce unwanted androgenic effects, the administration of a xenobiotic steroid with a favourable myotrophic–androgenic index should be considered. In the interim, at the very least it seems sensible to consider hormone replacement therapy to men in a catabolic state where there is a significant decrease in circulating testosterone associated with the chronic disease, for example, those with severe burn injuries or HIV-associated wasting.

For hormone replacement therapy, testosterone preparations are used in male hypogonadism and male hormonal contraception (where progestogens are administered to inhibit gonadotropin secretion). Mesterolone is also available for the treatment of male hypogonadism but it is seldom used, if at all. Oxymetholone and stanozolol, which induce the production of a C-1 esterase inhibitor, were used in the prevention and control of attacks of hereditary angioedema (except in pregnant women and prepubertal patients due to the risk of virilization) but the latter steroid has been recently withdrawn in the United Kingdom. Anabolic steroids also stimulate erythrocyte synthesis, which can be useful in the treatment of hypoplastic anaemias but their use in wealthy countries is likely to be limited with the relative recent availability of recombinant human erythropoietin and its analogues. In postmenopausal women, the treatment of osteoporosis with anabolic steroids, such as nandrolone decanoate, is not advocated given the success of oestrogen replacement and, more recently, with the introduction of the biphosphonates.

Anabolic steroids as performance enhancers in sport

The action of anabolic steroid in increasing skeletal muscle mass and strength in women is not questioned. Male and female athletes from the German Democratic Republic (GDR), from about 1972 onwards did exceptionally well in international events, being consistently in the top ranking of medal winners. Sporting performance among their female athletes, particularly in strength-dependent events, was

spectacular. Following the reunification of Germany in 1990, ground-breaking documental research was made by the former athlete, Mrs Brigitte Berendonk, and her husband Professor Werner Franke, who had succeeded in acquiring a number of highly classified scientific reports that had not been destroyed. These documents deal with the systematic state-sponsored programme of doping of athletes and included scientific reports, doctoral theses and a hand-written protocol book giving the times and dosage of administration of anabolic steroids to athletes. Several thousand athletes were treated with anabolic steroids every year, including adolescents of each sex. Particular emphasis was placed on the administration of anabolic steroids to women and adolescent girls, despite the virilizing effects, because of the rapid gains in sporting performance. It is important to note that the GDR scientists established (to themselves) that 'androgenic initiation' has permanent effects in girls and women, where increases in strength and performance do not return to pretreatment values after the drug is withdrawn. The current emphasis on out-of-competition drug testing is, therefore, of vital importance to help prevent doping during training followed by a period of drug elimination and then competition.

With respect to men, a most comprehensive review in 1976 of previous results concluded that there was little evidence for supraphysiological doses of testosterone or synthetic anabolic steroids having any appreciable effect on muscle size or strength in healthy men (Ryan, 1976). Even so, many of the studies reviewed had a lack of adequate control and standardization. Despite the debate in the scientific community as to the effectiveness of anabolic steroids as performance enhancers in men, male athletes and bodybuilders continued to use them, knowing from their own experimentation that they were effective. Conclusions from more recent reviews suggested that the administration of anabolic steroids could consistently result in significant increases in strength if male athletes satisfied certain criteria including the timing of doses and dietary factors (Wright, 1980; Haupt and Rovere, 1984; Alen and Hakkinen, 1987; Strauss and Yesalis, 1991). Then in 1996, Bhasin *et al*. (1996) in a very carefully designed study, proved beyond doubt that treatment with testosterone in supraphysiological doses (600 mg i.m. of testosterone enanthate for 10 weeks) increases muscle size and strength, and that with exercise these effects are augmented.

Subsequent work showed that increases in fat-free mass, muscle size, strength and power are highly dose-dependent and correlated with serum testosterone concentrations (Bhasin *et al*., 2001; Woodhouse *et al*., 2003) (Note: Strength is the maximum amount of force that can be exerted, for example, the heaviest weight that can be pushed away on a leg press, as opposed to power, which is the product of force and velocity, usually measured in watts, for example, the amount of weight that can be pressed away at speed, often repeatedly. Several track and field events demand explosive power, which depends on athletes first developing a solid strength base). The implications of these subsequent findings need to be emphasized to those concerned with antidoping in sport, in that an approximate doubling of the serum total and free concentrations of testosterone from the baseline values in eugonadal men over a 20-week period caused significant increases in strength and power (see the results reported by Bhasin *et al*. (2001), regarding the weekly regimen of 300 mg testosterone enanthate). Although the serum testosterone was measured 7 days after previous injection, which reflect the lowest values after administration, such androgen exposure is relatively small in the context of the regimens often written about in connection with bodybuilding. Even with respect to athletes, this dose is small compared with the amounts that some athletes may have been administering around 30 years ago, as Wright (1980) comments during that period that 'it is not uncommon for the dose level in national calibre athletes to exceed 1 mg/kg of body weight per day with a rather large number of individuals using two to four times that quantity.' Over a decade later, it is worth noting that Rogol and Yesalis (1992) remark that 'Endurance and sprint athletes use doses closer to those used medically for replacement levels', and the connotation is, therefore, that such athletes by that time recognized that modest drug-induced gains in strength and power may be all that is required to secure an advantage in these type of sports events. The use of smaller doses of anabolic steroids, particularly those formulated for daily administration (such as transdermal applications of testosterone as opposed to i.m. administered testosterone enanthate) would allow rapid elimination of a steroid in anticipation of a drug test. Out-of-competition testing should counter this strategy. Regardless of the above, it should be stressed that due to anabolic steroid administration being covert in athletics for obvious reasons, very little recent information has come to light regarding the doses of anabolic steroids used by elite athletes who choose to cheat.

Adverse effects

The undesirable effects arising from anabolic steroid administration (Table 3) have been extensively reviewed (Haupt and Rovere, 1984; Di Pasquale, 1990; Graham and Kennedy, 1990; Landry and Primos, 1990; Shahidi, 2001; Kicman and Gower, 2003b; James and Kicman, 2004). Typically, anabolic steroids are taken in cycles of about 6–12 weeks (the 'on period') followed by a variable period off the drugs, from 4 weeks to several months (the 'off period') in an attempt to reduce the likelihood of undesirable effects but some bodybuilders will take them almost continuously.

For clinical purposes, the administration of these drugs can be of therapeutic benefit and reasonably safe, with the physician making objective decisions based on the benefit/risk ratio in relation to a patient's condition. By contrast, for the purposes of enhancing performance in sport or for cosmetic purposes, usually because it is a clandestine activity, the athletes and bodybuilders are making subjective decisions regarding the effect these steroids are having on their health. Many probably have an attitude of personal invulnerability because they regard themselves as smart steroid users (Perry *et al*., 1990), their knowledge being based on reconnaissance of the considerable amount of popular literature (also in electronic form) written by steroid 'gurus', consultation of colleagues who are steroid users in the gym

Table 3 Adverse effects from anabolic steroid administration

Target	*Adverse effect*	*Comment*
Bone	Premature closure of the epiphysis in children	Stunting of linear growth
Breast	Atrophy in women	Gynaecomastia can be pronounced and painful; corrective surgery may be necessary. As some anabolics are known to be resistant to aromatization, other mechanisms need to be considered, such altered hepatic function causing an imbalance between androgens and estrogens
	Gynaecomastia and enlarged nipples in men	
Cardiovascular	Increases risk of thrombotic events such as myocardial infarction or stroke (raised LDL, lowered HDL and apolipoprotein-1, raised haematocrit (due to polycythaemia) and lowered plasma fibrinogen	The link between long-term anabolic steroid use and cardiovascular events remains to be clearly established but the evidence gathered is fairly compelling. This effect is probably underreported
	Cardiac damage (left ventricular hypertrophy, fibrosis and heart failure)	Heart disease may be potentiated by concomitant use of growth hormone or insulin (also misused for anabolic purposes)
	Sudden cardiac death	
CNS	Increased libido in men and women, which may be difficult to control	Psychological effects are unpredictable. Anabolic steroids are implicated in cases of violent behaviour ('roid rage') including, manslaughter and murder
	Hypomania (less severe form of mania)	
	Heightened irritability	Polypharmacy can increase the risk of violent criminality (Klotz *et al*., 2007)
	Increased aggression and hostility	
	Destructive Impulses	
	Self-destructive impulses	
	Withdrawal symptoms can include severe depression	
Hair	Hirsutism in women (conversion of vellus to terminal hair and male-body pattern of growth)	Hirsutism is at very best only partially reversible on cessation of administration.
	Acceleration of baldness in men; male-pattern baldness in women.	
Liver	Impaired function	Hepatoxicity is associated with 17α-alkylated steroids (orally active)
	Hepatic cholestasis (bile canal obstruction) causing jaundice	
	Peliosis hepatitis (blood-filled sacs in the liver)	For the liver function test, it is important to include GGT and CK, as ALT and AST can be raised naturally due to exercise
	Liver tumours (increased risk)	
Reproductive	Suppression of gonadal steroidogenesis	Recovery of fertility can take from months up to approximately 1 year after cessation of administration, depending on the extent of abuse
	Amennorhoea	
	Clitoral hypertrophy	Growth of the clitoris is irreversible
	Testicular atrophy	
	Disproportionate growth of the inner prostate	
	Masculanization of female foetus	
Skin	Cystic acne	This can be very severe on the chest, back and face
Vocal chords	Lengthened in women	Irreversible deepening of the voice can result in considerable distress
Other	Serious infection associated with injected drugs	Needle-exchange programmes are helping to address this problem
	Toxicity from unlicensed products?	Extent is unknown

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; GGT, γ-glutamyl transpeptidase; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

and their own personal experiences from experimentation. Furthermore, it may be perceived that athletes who fail a test show no obvious signs of ill-health, such as blatant gynaecomastia, severe steroid acne or hirsutism, and this may imply to others that the adverse effects of anabolic steroid use are exaggerated. These athletes could be exercising moderation in the doses they were administering, which should help to keep adverse effects to a minimum (Millar, 1994). Of note, however, is that many of the adverse effects can be difficult to recognize without a thorough medical examination (and patient–doctor confidentiality would have to be maintained) and other damaging effects are insidious where the athletes themselves will be unaware, such as the potential harmful changes to the cardiovascular system. Even so, it is important not to overstate the medical risks associated with anabolic steroid use (Hoffman and Ratamess, 2006) but to emphasize that the hazards to health are dependent on the sex, the dose, the duration of administration, whether hepatoxic 17α-alkylated steroids are being administered and the susceptibility of the individuals themselves to androgen exposure (likely to be dependent on genetic factors, age and lifestyle). The axiom, particularly among bodybuilders who can use excessively large amounts of steroids, that the 'more you take, the more you grow' should be accompanied with 'the more you may damage your health'. It is difficult to gauge the prevalence of severe adverse effects of what is an underground activity, and, moreover, it would be unethical to mimic the large dose regimens in controlled studies over prolonged periods of time to evaluate the risks to health. Notwithstanding, from the records of the doping programme in the former German Democratic Republic, nowhere did the GDR doctors record a damaging effect that was not described in the 'western' literature. These effects included the irreversible effects of virilization (masculanizing effects) in women and female adolescents, and life-threatening liver damage associated

with 17α-alkylated steroids (oral-turinabol was commonly administered), which sadly led to the death of the hammer thrower, Mr Detlef Gertsenberg, following postoperative complications.

Conflict of interest

The author states no conflict of interest.

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Testosterone dose-response relationships in healthy young men

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Bhasin, Shalender, Linda Woodhouse, Richard Casaburi, Atam B. Singh, Dimple Bhasin, Nancy Berman, Xianghong Chen, Kevin E. Yarasheski, Lynne Magliano, Connie Dzekov, Jeanne Dzekov, Rachelle Bross, Jeffrey Phillips, Indrani Sinha-Hikim, Ruoquing Shen, and Thomas W. Storer. Testosterone dose-response relationships in healthy young men. *Am J Physiol Endocrinol Metab* 281: E1172–E1181, 2001.—Testosterone increases muscle mass and strength and regulates other physiological processes, but we do not know whether testosterone effects are dose dependent and whether dose requirements for maintaining various androgen-dependent processes are similar. To determine the effects of graded doses of testosterone on body composition, muscle size, strength, power, sexual and cognitive functions, prostate-specific antigen (PSA), plasma lipids, hemoglobin, and insulin-like growth factor I (IGF-I) levels, 61 eugonadal men, 18–35 yr, were randomized to one of five groups to receive monthly injections of a long-acting gonadotropin-releasing hormone (GnRH) agonist, to suppress endogenous testosterone secretion, and weekly injections of 25, 50, 125, 300, or 600 mg of testosterone enanthate for 20 wk. Energy and protein intakes were standardized. The administration of the GnRH agonist plus graded doses of testosterone resulted in mean nadir testosterone concentrations of 253, 306, 542, 1,345, and 2,370 ng/dl at the 25-, 50-, 125-, 300-, and 600-mg doses, respectively. Fat-free mass increased dose dependently in men receiving 125, 300, or 600 mg of testosterone weekly (change +3.4, 5.2, and 7.9 kg, respectively). The changes in fat-free mass were highly dependent on testosterone dose ($P = 0.0001$) and correlated with log testosterone concentrations ($r = 0.73$, $P = 0.0001$). Changes in leg press strength, leg power, thigh and quadriceps muscle volumes, hemoglobin, and IGF-I were positively correlated with testosterone concentrations, whereas changes in fat mass and plasma high-density lipoprotein (HDL) cholesterol were negatively correlated. Sexual function, visual-spatial cognition and mood, and PSA levels did not change significantly at any dose. We conclude that changes in circulating testosterone concentrations, induced by GnRH agonist and testosterone administration, are associated with testosterone dose- and concentration-dependent changes in fat-free mass, muscle size, strength and power, fat mass, hemoglobin, HDL cholesterol, and IGF-I levels, in conformity with a single linear dose-response relationship. However, different androgen-dependent processes have different testosterone dose-response relationships.

sexual function; testosterone effects on muscle; cognitive function; plasma lipids; prostate-specific antigen; testosterone effects on insulin-like growth factor I; testosterone and hemoglobin

TESTOSTERONE regulates many physiological processes, including muscle protein metabolism, some aspects of sexual and cognitive functions, secondary sex characteristics, erythropoiesis, plasma lipids, and bone metabolism (7, 50). However, testosterone dose dependency of various androgen-dependent processes is not well understood (6). Administration of replacement doses of testosterone to hypogonadal men (10, 12, 30, 45, 49) and of supraphysiological doses to eugonadal men (9, 22–23, 26) increases fat-free mass, muscle size, and strength. Conversely, suppression of endogenous testosterone concentrations is associated with loss of fat-free mass and a decrease in fractional muscle protein synthesis (33). However, not known are whether testosterone effects on the muscle are dose dependent, or the nature of the testosterone dose-response relationships (6). Androgen receptors in most tissues are either saturated or downregulated at physiological testosterone concentrations (2, 18, 39, 50); this leads to speculation that there might be two separate dose-response curves: one in hypogonadal range, with

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maximal response at low normal testosterone concentrations, and a second in supraphysiological range, representing a separate mechanism of action (1). However, testosterone dose-response relationships for a range of androgen-dependent functions in humans have not been studied.

Animal studies suggest that different androgen-dependent processes have different androgen dose-response relationships (6, 8, 21). Sexual function in male mammals is maintained at serum testosterone concentrations that are at the lower end of the male range (3, 6, 8, 13, 21, 31). However, it is not known whether the low normal testosterone levels that normalize sexual function are sufficient to maintain muscle mass and strength, or whether the higher testosterone concentrations required to maintain muscle mass and strength might adversely affect plasma lipids, hemoglobin levels, and the prostate. This information is important for optimizing testosterone replacement regimens for treatment of hypogonadal men. Also, for the proposed use of testosterone in sarcopenia associated with aging (46, 47) and chronic illness (11, 27), it is important to know whether significant gains in muscle mass and strength can be achieved at testosterone doses that do not adversely affect plasma high-density lipoprotein (HDL) and prostate-specific antigen (PSA) levels.

Therefore, the primary objective of this study was to determine the dose dependency of testosterone's effects on fat-free mass and muscle performance. We hypothesized that changes in circulating testosterone concentrations would be associated with dose-dependent changes in fat-free mass, muscle strength, and power in conformity with a single linear dose-response relationship, and that the dose requirements for maintaining other androgen-dependent processes would be different. We treated young men with a long-acting gonadotropin-releasing hormone (GnRH) agonist to suppress endogenous testosterone secretion, and concomitantly also with one of five testosterone-dose regimens to create different levels of serum testosterone concentrations extending from subphysiological to the supraphysiological range. The lowest testosterone dose, 25 mg weekly, was selected because this dose had been shown to maintain sexual function in GnRH antagonist-treated men (37). The selection of the 600-mg weekly dose was based on the consideration that this was the highest dose that had been safely administered to men in controlled studies (9).

METHODS

This was a double-blind, randomized study consisting of a 4-wk control period, a 20-wk treatment period, and a 16-wk recovery period. Each participant provided informed consent, approved by the institutional review boards of Drew University and Harbor-UCLA Research and Education Institute.

Participants. The participants were healthy men, 18–35 yr of age, with prior weight-lifting experience and normal testosterone levels. These men had not used any anabolic agents and had not participated in competitive sports events in the preceding year, and they were not planning to participate in competitive events in the following year.

Randomization. Sixty-one eligible men were randomly assigned to one of five groups. All received monthly injections of a long-acting GnRH agonist to suppress endogenous testosterone production. In addition, *group 1* received 25 mg of testosterone enanthate intramuscularly weekly; *group 2*, 50 mg testosterone enanthate; *group 3*, 125 mg testosterone enanthate; *group 4*, 300 mg testosterone enanthate; and *group 5*, 600 mg testosterone enanthate. Twelve men were assigned to *group 1*, 12 to *group 2*, 12 to *group 3*, 12 to *group 4*, and 13 to *group 5*. Testosterone and GnRH agonist injections were administered by the General Clinical Research Center staff to assure compliance.

Nutritional intake. Energy and protein intakes were standardized at 36 $kcal \cdot kg^{-1} \cdot day^{-1}$ and 1.2 $g \cdot kg^{-1} \cdot day^{-1}$, respectively. The standardized diet was initiated 2 wk before treatment was started; dietary instructions were reinforced every 4 wk. The nutritional intake was verified by analysis of 3-day food records and 24-h food recalls every 4 wk by use of the Minnesota Nutritional Software.

Exercise stimulus. The participants were asked not to undertake strength training or moderate-to-heavy endurance exercise during the study. These instructions were reinforced every 4 wk.

Outcome measures. Body composition and muscle performance were assessed at baseline and during *week 20*. Fat-free mass and fat mass were measured by underwater weighing and dual-energy X-ray absorptiometry (DEXA, Hologic 4500, Waltham, MA). Total thigh muscle and quadriceps muscle volumes were measured by MRI scanning.

For estimation of total body water, the men ingested 10 g of ^2H_2O (10, 11), and plasma samples were drawn at −15, 0, 120, 180, and 240 min. We measured ^2H abundance in plasma by nuclear magnetic resonance spectroscopy (10, 11), with a correction factor of 0.985 for exchangeable hydrogen. We measured bilateral leg press strength by use of the one-repetition maximum (1-RM) method (11). A seated leg press exercise with pneumatic resistance (Keiser Sport, Fresno, CA) was used for this purpose. Subjects performed 5–10 min of leg cycling and stretching warm-up and received instruction and practice in lifting mechanics before performing progressive warm-up lifts leading to the 1-RM. Seat position and the ensuing knee and hip angles, as well as foot placement, were measured and recorded for use in subsequent testing. To ensure reliability in this highly effort-dependent test, the 1-RM score was reassessed within 7 days, but not sooner than 2 days, after the first evaluation. If duplicate scores were within 5%, the higher of the two values was accepted as the strength score. If the two tests differed by >5%, additional studies were conducted, ≥2 days apart but within 7 days, until the two highest scores were within 5%. No subject required >2 days of strength assessment.

We also measured leg power, because power in the lower extremity is strongly related to performance of functional activities in the elderly (4). The sarcopenia that accompanies aging is due in large part to a loss of the fast-twitch type II fibers and the coincident decrease in explosive force. Muscular power is important in performing such daily activities as rising from a chair, climbing stairs, and walking with speed (4). Leg power was measured with a previously validated (4, 5) Nottingham leg extensor power rig. Subjects performed 10–15 trials of right leg and hip extension, attempting to generate as much force as possible by accelerating the leg rig's weighted flywheel from rest. The power score (in watts) was taken as the highest value observed during these trials with evidence of a plateau. As with the strength tests, power

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measurements were preceded by a 5- to 10-min warm-up, stretching, and practice. The power tests were repeated within 7 days, but not sooner than 2 days, after the first tests to reduce the effect of familiarization. To minimize test-retest variability, the angle of knee flexion and the seat position were recorded and maintained constant across tests.

Sexual function was assessed by daily logs of sexual activity and desire that were maintained for 7 consecutive days at baseline and during treatment by use of a published instrument (13). Spatial cognition was assessed by a computerized checkerboard test (38) and mood by Hamilton's depression (20) and Young's mania scales (24).

Adverse experiences, blood counts and chemistries, PSA, plasma lipids, total and free testosterone, luteinizing hormone (LH), sex steroid-binding globulin (SHBG), and insulin-like growth factor I (IGF-I) levels were measured periodically during control and treatment periods. Serum total testosterone was measured by an immunoassay (8–11); free testosterone by equilibrium dialysis (43); LH, SHBG, and PSA by immunoradiometric assays (9–11); and IGF-I by acid-ethanol extraction and immunoassay (28). The sensitivities and intra- and interassay coefficients of variation for hormone assays were as follows: total testosterone (0.6 ng/dl), 8.2 and 13.2%; free testosterone (0.22 pg/ml), 4.2 and 12.3%; LH (0.05 U/l), 10.7 and 13.0%; SHBG (6.25 nmol/l), 4 and 6%; PSA (0.01 ng/ml), 5.0 and 6.4%; and IGF-I (80 ng/ml), 4 and 6%, respectively. These assays have been validated previously (8–11).

Statistical analyses. All variables were examined for their distribution characteristics. Variables not meeting the assumption of a normal distribution were log-transformed and retested. An ANOVA was used to compare change from baseline in outcome measures among the five groups. All outcome measures were analyzed using a paired *t*-test to detect a nonzero change from baseline within each group. $P < 0.05$ was considered statistically significant.

To describe the relationship between testosterone concentrations (T) and change in fat-free mass (Y) during testosterone administration, we tested three models: a linear model ($Y = a + bT$); a logarithmic model, $Y = a + b \cdot X$, where $X = \log(T)$, and a and b represent the intercept and slope, respectively; and a growth model, $Y = a/(1 + b \cdot e^{-k \cdot X})$. The logarithmic model provided the best fit for the data and was used to model the effects of testosterone concentrations on the change in other outcome variables. The correlations between testosterone concentrations and change in outcome variables are derived from this model. We also modeled the linear dependence of the change in outcome variables on testosterone dose by use of linear regression.

RESULTS

Participant characteristics. Of 61 men enrolled, 54 completed the study: 12 in *group 1*, 8 in *group 2*, 11 in *group 3*, 10 in *group 4*, and 13 in *group 5*. One man discontinued treatment because of acne; other subjects were unable to meet the demands of the protocol. The five groups did not significantly differ with respect to their baseline characteristics (Table 1).

Compliance. All evaluable subjects received 100% of their GnRH agonist injections, and only one man in the 125-mg group missed one testosterone injection.

Nutritional intake. Daily energy intake and proportion of calories derived from protein, carbohydrate, and fat were not significantly different among the five groups at baseline. There was no significant change in daily caloric, protein, carbohydrate, or fat intake in any group during treatment (data not shown).

Hormone levels. Serum total and free testosterone levels (Table 2), measured during *week 16*, 1 wk after the previous injection, were linearly dependent on the testosterone dose ($P = 0.0001$). Serum total and free testosterone concentrations decreased from baseline in men receiving the 25- and 50-mg doses and increased at 300- and 600-mg doses. Serum LH levels were suppressed in all groups. Serum SHBG levels decreased dose dependently at the 300- and 600-mg doses but did not change in other groups. Serum IGF-I concentrations increased dose dependently at the 300- and 600-mg doses (correlation between log testosterone level and change in IGF-I = 0.55, $P = 0.0001$). IGFBP-3 levels did not change significantly in any group.

Body composition. Fat-free mass, measured by underwater weighing, did not change significantly in men receiving the 25- or 50-mg testosterone dose, but it increased dose dependently at higher doses (Table 3). The changes in fat-free mass were highly dependent on testosterone dose ($P = 0.0001$) and correlated with log total testosterone concentrations during treatment ($r = 0.73$, $P = 0.0001$, see Fig. 2).

Changes in fat-free mass, measured by DEXA scan, were qualitatively similar to those obtained from underwater weighing (Table 3, Fig. 1). The measurements of fat-free mass by DEXA were highly correlated with values obtained from underwater weighing ($r = 0.94$, $P < 0.0001$).

Table 1. *Baseline characteristics of the participants*

GnRH Agonist + Testosterone Enanthate	+ 25 mg	+ 50 mg	+ 125 mg	+ 300 mg	+ 600 mg	*P* Value
Age, yr	28 ± 5	29 ± 5	28 ± 3	24 ± 5	25 ± 4	0.0583
Height, cm	175 ± 5	177 ± 9	178 ± 7	177 ± 7	175 ± 8	0.7230
Weight, kg	68.0 ± 8.4	77.0 ± 8.1	78.9 ± 10.6	78.4 ± 10.1	74.8 ± 12.5	0.1014
Body mass index, kg/m^2	23 ± 3	25 ± 3	25 ± 3	25 ± 3	25 ± 3	0.3680
Serum testosterone levels, nmol/l	593 ± 161	566 ± 220	553 ± 182	654 ± 157	632 ± 228	0.7093
Fat-free mass, kg	59.1 ± 6.7	65.1 ± 5.1	66.0 ± 7.2	67.3 ± 8.9	64.2 ± 8.0	0.1506
Leg press strength, kg	355.5 ± 103.8	407.8 ± 62.2	419.2 ± 86.2	439.8 ± 81.4	431.6 ± 99.3	0.2149
Hemoglobin, g/l	144 ± 12	151 ± 11	142 ± 9	144 ± 8	141 ± 8	0.1428
No. in group	12	12	12	12	13	

Values are means ± SD. GnRH, gonadotropin-releasing hormone.

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Table 2. *Serum total and free testosterone, LH, FSH, SHBG, and IGF-I levels*

Testosterone Dose	Baseline	*Week 16*	Change from Baseline	*P* vs. Zero Change
Testosterone (ng/dl) (overall ANOVA P = 0.0001)				
25 mg	593 ± 48	253 ± 66	−340 ± 85	0.0029
50 mg	566 ± 78	306 ± 58	−260 ± 64	0.0037
125 mg	553 ± 53	570 ± 75	57 ± 75	0.7425
300 mg	653 ± 50	1,345 ± 139	691 ± 143	0.0005
600 mg	632 ± 63	2,370 ± 150	1,737 ± 156	0.0001
Free testosterone (pg/ml) (overall ANOVA P = 0.0001)				
25 mg	62 ± 6	29 ± 5	−33 ± 8	0.0014
50 mg	57 ± 6	32 ± 3	−25 ± 5	0.0009
125 mg	49 ± 5	52 ± 8	3 ± 7	0.8601
300 mg	71 ± 7	138 ± 21	67 ± 18	0.0012
600 mg	64 ± 5	275 ± 30	211 ± 31	0.0001
LH (U/l) (overall ANOVA P = 0.8054)				
25 mg	3.5 ± 0.4	0.3 ± 0.1	−3.2 ± 0.4	0.0001
50 mg	3.8 ± 0.3	0.6 ± 0.3	−3.0 ± 0.4	0.0008
125 mg	3.4 ± 0.3	0.5 ± 0.1	−2.8 ± 0.4	0.0001
300 mg	3.7 ± 0.5	0.6 ± 0.1	−3.5 ± 0.5	0.0002
600 mg	3.3 ± 0.3	0.6 ± 0.4	−2.9 ± 0.4	0.0001
SHBG (nmol/l) (overall ANOVA P = 0.0001)				
25 mg	29.1 ± 2.9	28.5 ± 3.6	−0.6 ± 2.9	0.8497
50 mg	24.4 ± 3.4	21.1 ± 3.2	−3.3 ± 1.1	0.0202
125 mg	33.1 ± 4.2	28.9 ± 3.8	−4.2 ± 2.6	0.1410
300 mg	31.4 ± 3.8	22.4 ± 3.9	−9.1 ± 3.7	0.0348
600 mg	40.1 ± 4.9	20.6 ± 3.2	−19.5 ± 2.8	0.0001
IGF-I (ng/ml) (overall ANOVA P = 0.0001)				
25 mg	268 ± 26	261 ± 35	−7 ± 19	0.7462
50 mg	246 ± 14	225 ± 12	−20 ± 10	0.0797
125 mg	299 ± 24	282 ± 31	−18 ± 17	0.3284
300 mg	314 ± 24	388 ± 30	74 ± 28	0.0272
600 mg	227 ± 20	304 ± 21	77 ± 13	0.0001

Values on each day represent the mean (±SE) of all available values on that day. However, the change represents the difference between paired values only. Treatment values represent the *day 113* (*week 16*) values, obtained 1 wk after the previous testosterone injection. We used *week 16* rather than *week 20* values because *week 20* values were not always drawn exactly 1 wk after the previous injection. LH and FSH, luteinizing and follicle-stimulating hormones, respectively; SHBG, sex hormone-binding globulin; IGF-I, insulin-like growth factor I. To convert total testosterone levels to nmol/l, multiply by 0.03467. To convert free testosterone levels to pg/ml, multiply by 3.467.

To determine whether the apparent changes in fat-free mass by DEXA scan and underwater weighing represented water retention, we measured total body water and compared the ratios of total body water to fat-free mass before and after treatment in each group. The ratios of total body water to fat-free mass by underwater weighing did not significantly change with treatment in any treatment group (Table 3), indicating that the apparent increase in fat-free mass measured by underwater weighing did not represent water retention in excess of that associated with protein accretion.

Fat mass, measured by underwater weighing, increased significantly in men receiving the 25- and 50-mg doses but did not change in men receiving the higher doses of testosterone (Table 3, Fig. 1). There was an inverse correlation between change in fat mass by underwater weighing and log testosterone concentrations ($r = -0.60$, $P = 0.0001$, Fig. 2).

Muscle size. The thigh muscle volume and quadriceps muscle volume did not significantly change in men receiving the 25- or 50-mg doses but increased dose dependently at higher doses of testosterone (Table 4, Fig. 1). The changes in thigh muscle and quadriceps muscle volumes correlated with log testosterone levels during treatment ($r = 0.66$, $P = 0.0001$, and $r = 0.55$, $P = 0.0001$, respectively, Fig. 2).

Muscle performance. The leg press strength did not change significantly in the 25- and 125-mg-dose groups but increased significantly in those receiving the 50-, 300-, and 600-mg doses (Table 5). The changes in leg press strength correlated with log testosterone levels during treatment ($r = 0.48$, $P = 0.0005$, Fig. 2) and changes in muscle volume ($r = 0.54$, $P = 0.003$) and fat-free mass ($r = 0.74$, $P < 0.0001$).

Table 3. *Body composition analysis*

Testosterone Dose	Baseline	*Week 20*	Change from Baseline	*P* vs. Zero Change
Fat-free mass (kg) by underwater weighing (overall ANOVA P = 0.0001)				
25 mg	61.1 ± 2.7	58.1 ± 1.7	−1.0 ± 0.5	0.0695
50 mg	66.1 ± 2.5	65.7 ± 2.0	+0.6 ± 0.4	0.1324
125 mg	66.0 ± 2.1	67.9 ± 2.7	+3.4 ± 0.8	0.0024
300 mg	66.9 ± 2.4	72.4 ± 2.8	+5.2 ± 0.8	0.0001
600 mg	64.2 ± 2.2	72.1 ± 2.4	+7.9 ± 0.6	0.0001
Fat mass (kg) by underwater weighing (overall ANOVA P = 0.0001)				
25 mg	8.3 ± 1.4	11.3 ± 1.6	+3.1 ± 0.7	0.0014
50 mg	10.9 ± 1.4	14.3 ± 1.7	+3.5 ± 1.0	0.0096
125 mg	12.2 ± 2.0	10.9 ± 2.1	+0.01 ± 0.5	0.9820
300 mg	11.4 ± 1.6	10.9 ± 1.7	−0.5 ± 0.6	0.4134
600 mg	9.4 ± 1.9	8.8 ± 1.9	−1.1 ± 0.7	0.1132
Fat-free mass (kg) by DEXA scan (overall ANOVA P = 0.0001)				
25 mg	53.6 ± 1.8	53.4 ± 2.0	+0.4 ± 0.3	0.2198
50 mg	58.6 ± 2.3	59.2 ± 2.5	+1.1 ± 0.9	0.2313
125 mg	60.1 ± 2.1	63.1 ± 2.3	+2.9 ± 0.8	0.0054
300 mg	59.0 ± 2.7	64.3 ± 2.2	+5.5 ± 0.7	0.0001
600 mg	57.4 ± 1.9	66.3 ± 2.4	+8.9 ± 0.8	0.0001
Fat mass (kg) by DEXA scan (overall ANOVA P = 0.0004)				
25 mg	10.0 ± 1.8	13.7 ± 1.4	+3.6 ± 1.5	0.0326
50 mg	15.4 ± 1.2	17.9 ± 1.2	+2.6 ± 1.0	0.0324
125 mg	15.2 ± 2.0	15.2 ± 1.9	−0.3 ± 0.8	0.6882
300 mg	16.3 ± 1.2	15.41 ± 1.5	−0.9 ± 0.6	0.1834
600 mg	14.2 ± 1.9	12.0 ± 1.5	−2.0 ± 0.7	0.0141
Ratio of total body water to fat-free mass (percent) (overall ANOVA for change from baseline, P = 0.270)				
25 mg	62.7 ± 2.7	63.7 ± 2.1	+1.1 ± 2.4	
50 mg	62.0 ± 1.9	63.8 ± 2.4	+2.0 ± 2.0	
125 mg	67.0 ± 1.7	63.5 ± 3.0	−3.8 ± 1.6	
300 mg	61.6 ± 2.7	64.6 ± 3.1	+2.1 ± 2.5	
600 mg	65.3 ± 2.4	67.4 ± 2.8	+2.5 ± 1.7	

Values on each day represent the mean (±SE) of all available values on that day. However, the change represents the difference between paired values only. Ratios of total body water assessed by deuterium water dilution to fat-free mass measured by underwater weighing were calculated for each subject and averaged across subjects within each group. DEXA, dual-energy X-ray absorptiometry.

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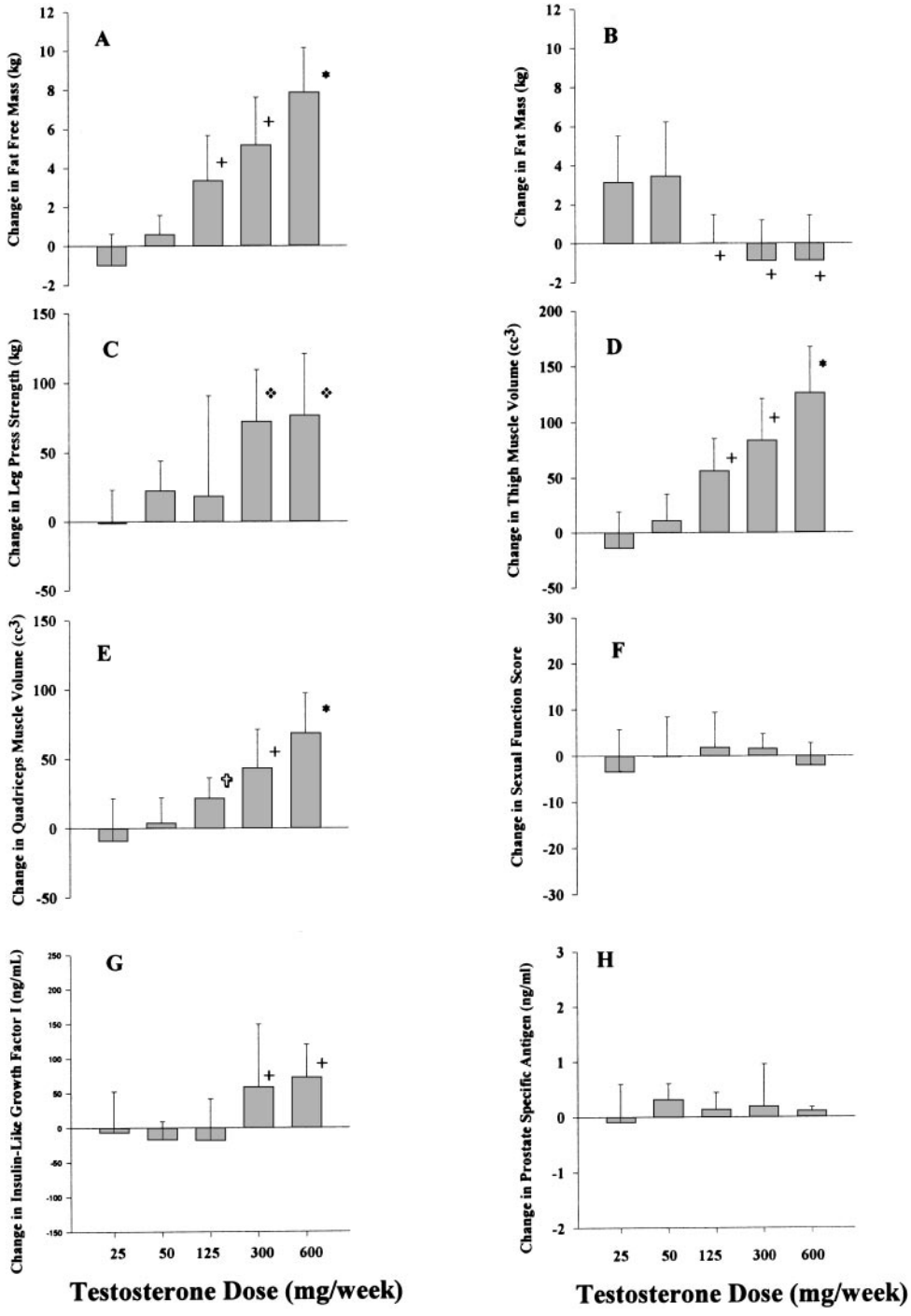

Fig. 1. Change in fat-free mass (*A*), fat mass (*B*), leg press strength (*C*), thigh muscle volume (*D*), quadriceps muscle volume (*E*), sexual function (*F*), insulin-like growth factor I (*G*), and prostate-specific antigen (*H*). Data are means ± SE. *Significant differences from all other groups ($P < 0.05$); ❖ significant difference from 25-, 50-, and 125-mg doses ($P < 0.05$); +significant difference from 25- and 50-mg doses ($P < 0.05$); and ⇧ significant difference from 25-mg dose ($P < 0.05$).

Leg power, measured by the Nottingham leg rig, did not change significantly in men receiving the 25-, 50-, and 125-mg doses of testosterone weekly, but it increased significantly in those receiving the 300- and 600-mg doses. The increase in leg power correlated with log testosterone concentrations ($r = 0.39$, $P = 0.0105$, Fig. 2) and changes in fat-free mass ($r = 0.30$, $P = 0.0392$) and muscle strength ($r = 0.42$, $P = 0.0020$).

Behavioral measures. The scores for sexual activity and sexual desire measured by daily logs did not change significantly at any dose. Similarly, visual-spatial cognition (Table 6) and mood, as assessed by Hamilton's depression and Young's manic scales (data not shown), did not change significantly in any group.

Adverse experiences and safety measures. Hemoglobin levels decreased significantly in men receiving the 50-mg dose but increased at the 600-mg dose; the changes in hemoglobin were positively correlated with testosterone concentrations ($r = 0.66$, $P = 0.0001$) (Table 7). Changes in plasma HDL cholesterol, in contrast, were negatively dependent on testosterone dose ($P = 0.0049$) and correlated with testosterone concentrations ($r =$

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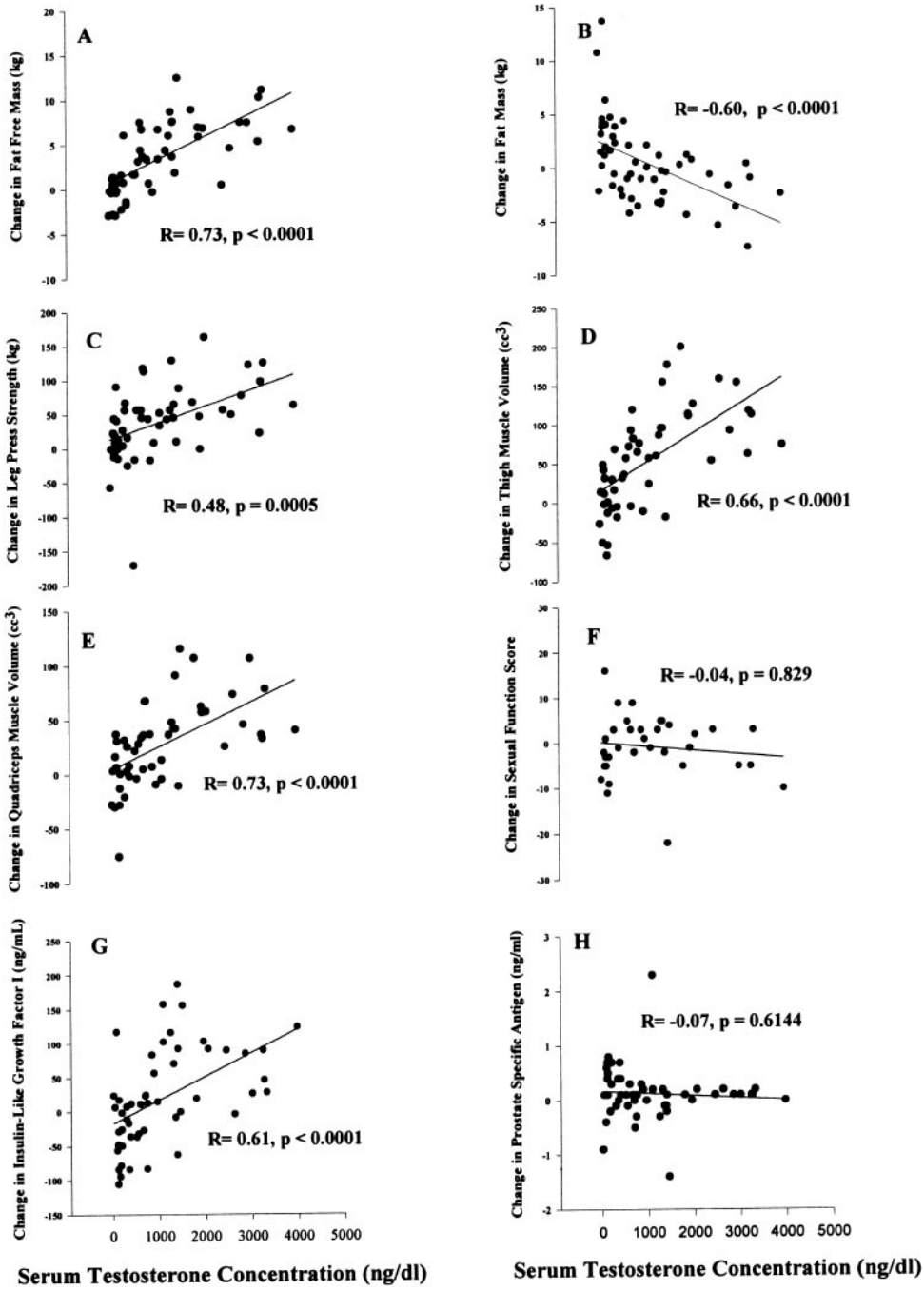
A
Change in Fat Free Mass (kg)
R= 0.73, p < 0.0001
B
Change in Fat Mass (kg)
R= -0.60, p < 0.0001
C
Change in Leg Press Strength (kg)
R= 0.48, p = 0.0005
D
Change in Thigh Muscle Volume (cc3)
R= 0.66, p < 0.0001
E
Change in Quadriceps Muscle Volume (cc3)
R= 0.73, p < 0.0001
F
Change in Sexual Function Score
R= -0.04, p = 0.829
G
Change in Insulin-Like Growth Factor I (ng/mL)
R= 0.61, p < 0.0001
H
Change in Prostate Specific Antigen (ng/ml)
R= -0.07, p = 0.6144
0 1000 2000 3000 4000 5000
Serum Testosterone Concentration (ng/dl)
Serum Testosterone Concentration (ng/dl)

Fig. 2. Relationship between serum testosterone concentrations (T) during treatment (*week 16*) and change in fat-free mass (*A*), fat mass (*B*), leg press strength (*C*), thigh muscle volume (*D*), quadriceps muscle volume (*E*), sexual function (*F*), insulin-like growth factor I (*G*), and prostate-specific antigen (*H*). The correlation coefficient, *r*, was calculated using the logarithmic model, $Y = a + b \cdot X$, where X = log (T), and *a* and *b* represent the intercept and slope.

−0.40, $P = 0.0054$). Total cholesterol, plasma low-density lipoprotein cholesterol, and triglyceride levels did not change significantly at any dose. Serum PSA, creatinine, bilirubin, alanine aminotransferase, and alkaline phosphatase did not change significantly in any group, but aspartate aminotransferase decreased significantly in the 25-mg group. Two men in the 25-mg group, five in the 50-mg group, three in the 125-mg group, seven in the 300-mg group, and two in the 600-mg group developed acne. One man receiving the 50-mg dose reported decreased ability to achieve erections.

DISCUSSION

GnRH agonist administration suppressed endogenous LH and testosterone secretion; therefore, circulating testosterone concentrations during treatment were proportional to the administered dose of testosterone enanthate. This strategy of combined administration of GnRH agonist and graded doses of testosterone enanthate was effective in establishing different levels of serum testosterone concentrations among the five treatment groups. The different levels of circulating testosterone concentrations created by this regimen were associated with dose- and concentration-

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Table 4. *Thigh and quadriceps muscle volume measured by MRI*

Testosterone Dose	Baseline	*Week 20*	Change from Baseline	*P* vs. zero change
Thigh muscle volume (overall ANOVA P = 0.0001)				
25 mg	753 ± 46	739 ± 44	−14 ± 10	0.1958
50 mg	833 ± 53	844 ± 58	11 ± 8	0.2332
125 mg	890 ± 49	966 ± 60	56 ± 10	0.0004
300 mg	849 ± 39	933 ± 39	84 ± 12	0.0001
600 mg	802 ± 45	928 ± 48	126 ± 12	0.0001
Quadriceps muscle volume (overall ANOVA P = 0.0001)				
25 mg	436 ± 30	427 ± 27	−9 ± 9	0.3524
50 mg	489 ± 34	493 ± 36	4 ± 7	0.5889
125 mg	508 ± 29	546 ± 36	21 ± 5	0.0027
300 mg	497 ± 25	540 ± 22	43 ± 9	0.0008
600 mg	472 ± 27	540 ± 31	68 ± 8	0.0001

Values (in cm^3) on each day represent the mean (±SE) of all available values on that day. However, the change represents the difference between paired values only.

dependent changes in fat-free mass, fat mass, thigh and quadriceps muscle volume, muscle strength, leg power, hemoglobin, circulating IGF-I, and plasma HDL cholesterol. Serum PSA levels, sexual desire and activity, and spatial cognition did not change significantly at any dose. The changes in fat-free mass, muscle volume, leg press strength and power, hemoglobin, and IGF-I were positively correlated, whereas changes in plasma HDL cholesterol and fat mass were negatively correlated with testosterone dose and total and free testosterone concentrations during treatment.

The compliance with the treatment regimen was high. The participants received 100% of their scheduled GnRH agonist, and 99% of testosterone injections. Serum LH levels were suppressed in all men, demonstrating the effectiveness of GnRH agonist treatment. The treatment regimen was well tolerated. There were no significant changes in PSA or liver enzymes at any dose. However, long-term effects of androgen administration on the prostate, cardiovascular risk, and behavior are unknown.

Table 5. *Change in measures of muscle performance*

Testosterone Dose	Baseline	Treatment	Change from Baseline	*P* vs. Zero Change
Leg press strength (kg) (overall ANOVA P = 0.0003)				
25 mg	355.5 + 31.3	354.2 ± 27.9	−1.2 ± 7.4	0.8701
50 mg	407.8 + 22.0	430.5 ± 22.3	+22.7 ± 7.6	0.0204
125 mg	419.2 ± 24.4	444.6 ± 32.2	+18.4 ± 10.0	0.4195
300 mg	439.8 ± 25.7	525.5 ± 24.9	+72.2 ± 12.4	0.0004
600 mg	431.6 + 27.6	508.1 ± 28.1	+76.5 ± 12.2	0.0001
Leg power (W) (overall ANOVA P = 0.0419)				
25 mg	183.6 ± 10.6	188.9 ± 12.9	5.3 ± 8.4	0.5429
50 mg	234.4 ± 14.2	249.6 ± 17.8	15.2 ± 15.0	0.3468
125 mg	253.8 ± 20.6	265.6 ± 25.2	8.5 ± 15.3	0.5935
300 mg	233.8 ± 20.2	272.4 ± 27.8	38.6 ± 9.4	0.0033
600 mg	212.4 ± 11.0	256.2 ± 13.8	48.1 ± 11.8	0.0015

Values on each day represent the mean (±SE) of all available values on that day. However, the change represents the difference between paired values only.

Table 6. *Change in scores for sexual activity, sexual desire, and spatial cognition*

Testosterone Dose	Baseline	Treatment	Change from Baseline	*P* vs. zero change
Sexual activity scores (overall ANOVA P = 0.7842)				
25 mg	10.7 ± 1.7	8.2 ± 2.9	−2.5 ± 3.2	0.4729
50 mg	14.1 ± 2.1	13.7 ± 1.8	−0.4 ± 2.8	0.9017
125 mg	9.8 ± 2.7	12.0 ± 2.9	2.2 ± 3.1	0.5151
300 mg	11.6 ± 1.6	12.0 ± 1.9	0.7 ± 0.9	0.4761
600 mg	16.1 ± 3.7	15.6 ± 0.5	0.7 ± 2.2	0.7891
Intensity of sexual desire scores (overall ANOVA P = 0.477)				
25 mg	1.9 ± 0.1	1.3 ± 0.4	−0.6 ± 0.4	0.2253
50 mg	2.3 ± 0.1	2.2 ± 0.3	−0.0 ± 0.3	0.9615
125 mg	2.1 ± 0.1	2.0 ± 0.3	−0.1 ± 0.4	0.9078
300 mg	2.2 ± 0.2	2.4 ± 0.2	0.1 ± 0.1	0.3559
600 mg	2.7 ± 0.2	2.2 ± 0.1	0.2 ± 0.2	0.4075
Spatial cognition scores				
1. No. of trial levels on the checkerboard test that the participant reached before the test was terminated (overall ANOVA P = 0.235)				
25 mg	6.8 ± 0.3	6.4 ± 0.3	−0.4 ± 0.3	0.284
50 mg	6.7 ± 0.3	6.7 ± 0.3	0.3 ± 0.3	0.284
125 mg	6.6 ± 0.3	6.6 ± 0.2	0.0 ± 0.4	1.0
300 mg	7.3 ± 0.2	6.7 ± 0.2	−0.6 ± 0.3	0.103
600 mg	6.6 ± 0.2	6.9 ± 0.2	0.3 ± 0.3	0.278
2. No. of checkerboard squares correctly marked in all trials (overall ANOVA P = 0.6309)				
25 mg	28.6 ± 2.2	30.4 ± 2.1	1.8 ± 2.1	0.4272
50 mg	30.0 ± 2.3	34.7 ± 4.9	2.7 ± 3.5	0.5236
125 mg	27.3 ± 3.0	28.1 ± 2.2	0.9 ± 3.8	0.7292
300 mg	32.6 ± 2.1	33.3 ± 1.8	0.7 ± 3.1	0.8241
600 mg	26.7 ± 2.7	32.5 ± 2.1	5.8 ± 2.2	0.0265

Values are means ± SE.

Serum testosterone levels were measured 7 days after previous injection; they reflect the lowest testosterone levels after an injection. Testosterone concentrations were higher at other time points. Weekly injections of testosterone enanthate are associated with fluctuations in testosterone levels (44). Although nadir testosterone concentrations were highly correlated with testosterone enanthate dose, it is possible that sustained testosterone delivery by a patch or gel might reveal different dose-response relationships, particularly with respect to hemoglobin and HDL cholesterol (19).

There were no significant changes in overall sexual activity or sexual desire in any group, including those receiving the 25-mg dose. Testosterone replacement of hypogonadal men improves frequency of sexual acts and fantasies, sexual desire, and response to visual erotic stimuli (3, 13, 15, 17, 31, 41). Our data demonstrate that serum testosterone concentrations at the lower end of male range can maintain some aspects of sexual function (3, 13). Testosterone has been shown to regulate nitric oxide synthase activity in the cavernosal smooth muscle (32), and it is possible that optimum penile rigidity might require higher testosterone levels than those produced by the 25-mg dose.

This study demonstrates that an increase in circulating testosterone concentrations results in dose-de-

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Table 7. *Changes in hemoglobin, plasma HDL cholesterol, and PSA*

Testosterone Dose	Baseline	Week 20	Change from Baseline	*P* vs. Zero Change
Hemoglobin (g/l), (overall ANOVA P = 0.0001)				
25 mg	143.5 ± 3.5	139.0 ± 2.5	−5.2 ± 3.5	0.1759
50 mg	150.8 ± 3.3	146.6 ± 2.0	−7.4 ± 2.3	0.0153
125 mg	141.9 ± 2.6	146.1 ± 3.1	2.5 ± 2.4	0.3061
300 mg	143.5 ± 2.2	149.6 ± 3.1	6.1 ± 2.9	0.0639
600 mg	141.5 ± 2.3	155.7 ± 2.2	14.2 ± 2.0	0.0001
PSA (ng/ml), (overall ANOVA P = 0.5290)				
25 mg	1.0 ± 0.2	1.0 ± 0.2	−0.1 ± 0.2	0.6870
50 mg	0.8 ± 0.1	1.1 ± 0.2	0.3 ± 0.1	0.0186
125 mg	0.7 ± 0.1	0.8 ± 0.1	0.1 ± 0.1	0.1721
300 mg	0.7 ± 0.1	0.9 ± 0.3	0.2 ± 0.2	0.4525
600 mg	0.5 ± 0.1	0.7 ± 0.1	0.1 ± 0.0	0.0010
Plasma HDL cholesterol (mg/dl) (overall ANOVA P = 0.0049)				
25 mg	46 ± 3	51 ± 4	+4.5 ± 2.6	0.1202
50 mg	48 ± 3	47 ± 5	−0.7 ± 4.0	0.8653
125 mg	48 ± 2	43 ± 3	−4.0 ± 1.7	0.0476
300 mg	47 ± 3	41 ± 2	−5.7 ± 2.8	0.0690
600 mg	43 ± 2	34 ± 2	−8.4 ± 1.8	0.0005

Values on each day represent the mean (±SE) of all available values on that day. However, the change from baseline represents the difference between paired values only. PSA, prostate-specific antigen; HDL, high-density lipoprotein.

pendent increases in fat-free mass, muscle size, strength, and power. The relationships between circulating testosterone concentrations and changes in fat-free mass and muscle size conform to a single log-linear dose-response curve. Our data do not support the notion of two separate dose-response curves reflecting two independent mechanisms of testosterone action on the muscle. Forbes et al. (22) predicted 25 years ago that the muscle mass accretion during androgen administration is related to the cumulative androgen dose, the product of daily dose and treatment duration. Our data are consistent with Forbes's hypothesis of a linear relationship between testosterone dose and lean mass accretion; however, we do not know whether increasing the treatment duration would lead to further gains in muscle mass.

In addition, we do not know whether responsiveness to testosterone is attenuated in older men. Testosterone dose-response relationships might be modulated by other muscle growth regulators, such as nutritional status, exercise and activity level, glucocorticoids, thyroid hormones, and endogenous growth hormone secretory status.

Serum PSA levels decrease after androgen withdrawal, and testosterone replacement of hypogonadal men increases PSA levels into the normal range (16, 34). We did not find significant changes in PSA at any dose, indicating that the lowest dose of testosterone maintained PSA levels. We did not measure prostate volume in this study; therefore, we do not know whether prostate volume exhibits the same relationship with testosterone dose as PSA levels.

Hemoglobin levels changed significantly in relation to testosterone dose and concentration. Testosterone regulates erythropoiesis through its effects on erythropoietin and stem cell proliferation (14, 35, 40). Although modest increments in hemoglobin might be beneficial in androgen-deficient men with chronic illness who are anemic, marked increases in hemoglobin levels could increase the risk of cerebrovascular events (25) and hypertension (42).

Although men, on average, perform better on tests of spatial cognition than women, testosterone replacement has not been consistently shown to improve spatial cognition in hypogonadal men (1, 29, 48). We did not find changes in spatial cognition at any dose. The effect size of gender differences in spatial cognition is small; it is possible that our study did not have sufficient power to detect small differences. We cannot exclude the possibility that gender differences in spatial cognition might be due to organizational effects of testosterone and might not respond to changes in testosterone levels in adult men.

Although mean change in fat-free mass and muscle size correlated with testosterone dose and concentration, there was considerable heterogeneity in response to testosterone administration within each group. These individual differences in response to androgen administration might reflect differences in activity level, testosterone metabolism, nutrition, or polymorphisms in androgen receptor, myostatin, 5-α-reductase, or other muscle growth regulators.

Our data demonstrate that different androgen-dependent processes have different testosterone dose-response relationships. Some aspects of sexual function and spatial cognition, and PSA levels, were maintained by relatively low doses of testosterone in GnRH agonist-treated men and did not increase further with administration of higher doses of testosterone. In contrast, graded doses of testosterone were associated with dose and testosterone concentration-dependent changes in fat-free mass, fat mass, muscle volume, leg press strength and power, hemoglobin, IGF-I, and plasma HDL cholesterol. The precise mechanisms for the tissue- and function-specific differences in testosterone dose dependence are not well understood (36). Although only a single androgen receptor protein is expressed in all androgen-responsive tissues, tissue specificity of androgen action might be mediated through combinatorial recruitment of tissue-specific coactivators and corepressors (36).

Testosterone doses associated with significant gains in fat-free mass, muscle size, and strength were associated with significant reductions in plasma HDL concentrations. Further studies are needed to determine whether clinically significant anabolic effects of testosterone can be achieved without adversely affecting cardiovascular risk. Selective androgen receptor modulators that preferentially augment muscle mass and strength, but only minimally affect prostate and cardiovascular risk factors, are desirable (36).

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THE ABSORPTION, DISTRIBUTION AND EXCRETION OF ANABOLIC AGENTS[1]

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Summary

The metabolic fate of the anabolic agents, diethylstilbestrol, hexestrol, trenbolone acetate, zeranol and the endogenous steroids are discussed under the headings absorption, distribution and excretion. There is an optimum concentration of anabolic agent in the systemic circulation that results in a maximum increase in growth rate of farm animals. This optimum blood concentration should be maintained over a long period. However, there is rapid metabolism and excretion of anabolic agents with short half-lives in blood, and metabolic clearance rate equals entry rate. The rate of absorption of the agent, which is determined by formulation and site of administration, is most important and is best achieved by the use of slow release implants. The pattern of exponential absorption from compressed pellets of single anabolic agents is not ideal, and a more constant payout of drug, in particular estradiol-17β, is best achieved in combined preparations of agents or from silicone rubber implants impregnated with the agent. The high metabolic clearance rate and rapid excretion of anabolic substances influences the distribution of residues. Outside the site of administration, less than 1% of the administered dose is present in the animal. The lowest concentrations of residues are found in muscle and fat, higher concentrations are present in liver and kidney and the highest concentrations are in the bile, urine and feces.

(Key Words: Anabolic Agents, Residues, Farm Animals, Implants, Formulation.)

Introduction

Many factors influence the fate of anabolic agents in farm animals including: (a) absorption as determined by the site of administration and formulation of the agent; (b) distribution within the various body tissues or the disposition of the agent; (c) metabolism of the agent and (d) excretion of the agent and its metabolism.

Although the exact target tissues and intracellular receptors for anabolic agents have not been clearly identified, the pituitary gland and the muscle cell are probably the most important, while the metabolism of the agent occurs in the blood, liver and kidney. Anabolic agents, through their action on the pituitary gland, may act indirectly at the muscle cell by changing the concentrations of other endogenous anabolic and catabolic hormones, e.g., growth hormone, insulin, prolactin, thyroxine, triiodothyronine and corticosteroids.

Anabolic agents pass freely into and out of cells. Within the cells of the target tissues they occupy specific receptors and their anabolic activity is probably proportional to the number of occupied binding sites. When all of the sites are occupied, the receptors are saturated and an increase in the concentration of agent has no further effect. Thus, there is an optimum or "threshold" cell concentration of the anabolic agents that results in maximum cell response and, ultimately, maximum increase in physiologic response. Although this optimum intracellular concentration is unknown, it is assumed to be proportional to the concentration of agent in the surrounding blood. Thus, if the active agent is maintained at a concentration in the circulation that maintains the threshold concentration in the target cells, the result is optimum growth performance in farm animals. Thus, the opti-

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mum drug delivery system is one that maintains the concentration of anabolic agent at the threshold concentration throughout the treatment period. When the threshold concentration is exceeded excessive amounts of the agent will be absorbed, and there may even be undesirably high residues in edible tissues. Such a situation has occurred in Europe, where veal calves were illegally given massive doses of diethylstilbestrol (DES) by im injection.

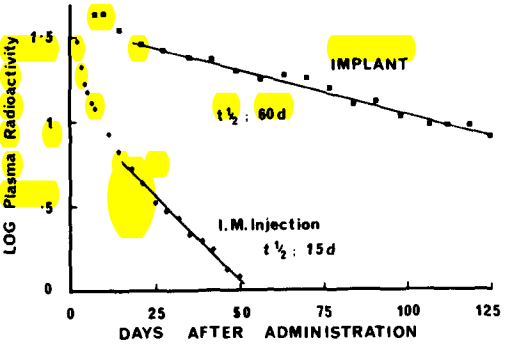

Figure 1. Absorption of trenbolone acetate by steers. Steers were given 300 mg ^{3}H trenbolone acetate either by implant in the ear (—■—■—) or by an im injection dissolved in groundnut oil (— ◆— ◆—). The total radioactivity in plasma was measured and the half-lives calculated from the slope of the lines shown. Data from Roussel-Uclaf, Paris and Pottier et al. (1975).

Absorption of Anabolic Agent

Preparations of anabolic agents can be classified according to the following scheme:

Formulation	Route of administration
Feed additive	Oral
Suspension — oil, microcrystalline	Im injection or sc injection
Compressed pellets, impregnated silicone rubber	Sc implant

Feed additives have the advantage that their regular intake maintains constant concentrations of agent in the circulation. However, the choice of agents is limited because most are metabolized either in the gut or rumen or during their initial absorption via the portal system in the liver. Only the synthetic agent, melengestrol acetate for ruminants in the United States, is still used as a feed additive.

Injectable suspensions of anabolic agents, either oil based or microcrystalline, are frequently used for therapeutic purposes, but they are less suitable for use as growth promoters because they are absorbed too rapidly from their injection site. For example, the half-life of a 300 mg im injection of trenbolone acetate is 15 d in cattle (figure 1).

The rate of absorption of trenbolone acetate given by injection is exponential (figure 1), and thus it is not generally possible to maintain a threshold concentration for a long period. Initially plasma concentration greatly exceeds the threshold concentration, and then falls too rapidly to be effective. However, the im injection of lipophilic long chain esters of anabolic agents, such as esters of 19-nortestosterone, prolongs the absorption time (Johndorf and Moss, 1978) and these preparations have been used in horses.

Compressed pellets and im injections of single anabolic agents exhibit similar exponential absorption kinetics, although their rate of dissolution is much slower. For example, the effective half-life of trenbolone acetate is 15 d for the injected form in oil, but is 60 d for the implant (figure 1). Implants of trenbolone acetate, zeranol, DES and hexestrol are widely used, and their effect on growth promotion persists for 2 to 4 mo after administration (Heitzman, 1979).

When compressed pellets are used containing a mixture of estradiol and a second steroid (trenbolone acetate, testosterone or progesterone) the absorption of estradiol from these combined implants is delayed even further and absorption maintained at a near constant rate for several months (Harrison, 1981). Plasma concentrations of estradiol in steers implanted with single or combined preparations of 140 mg trenbolone acetate and 20 mg estradiol are shown in figure 2. The delayed absorption of estradiol in the combined preparation was attributed to a physical interaction between the two steroids, because the absorption of estradiol placed separately in the opposite ear to trenbolone acetate was not different from the absorption of a single implant of estradiol (Heitzman et al., 1981). The same effect was observed in sheep given preparations of estradiol with trenbolone, progesterone or testosterone (Harrison, 1981). In steers treated with combined implants the increased concentrations of estradiol in plasma for the

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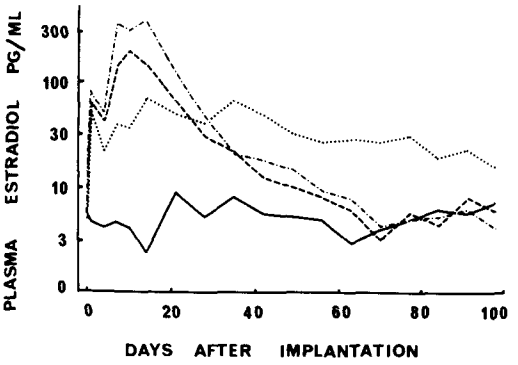

Figure 2. Estradiol in plasma of implanted steers. Concentrations of estradiol-17β in plasma of: (1) control steers (——); (2) steers treated with estradiol (– – –); (3) steers treated with trenbolone acetate and estradiol in separate ears (–•–•–) and (4) steers treated with a mixture of trenbolone acetate and estradiol in one ear (••••). Plasma samples were taken from the jugular vein ipsilateral to the estradiol implant. Data from Heitzman et al. (1981).

longer period resulted in greater improvements in live weight gain (figure 3), feed conversion effiiency and carcass weights (Heitzman et al., 1981).

The sustained slow release of anabolic agents has been further developed by the introduction of a new delivery system for estradiol. This system uses silicone rubber implants impregnated with estradiol. Absorption rate of estradiol is proportional to the surface area of the silastic implant (Wagner et al., 1979). It is claimed by the manufacturer that these implants can be used for 400 d in steers. The relationship between growth responses in steers and average daily amount of agent absorbed over a 200 d trial period is shown in figure 4. The data suggest that the average entry rate of estradiol into a steer for optimum performance is about 55 μg/d. The entry rate of estradiol slowly decreased throughout the treatment period; e.g., it was 45 μg/d on d 84 and 34 μg/d on d 196 after implantation (Wagner et al., 1979). Turner et al. (1981) reported that steers given a single implant delivering an average of 47 μg estradiol/d gained 8% more weight per day than untreated steers during a 499 d trial.

Metabolism and Disposition of Anabolic Agents

The metabolism of anabolic agents has been described by Rico (1983). Anabolic agents are generally metabolized and the

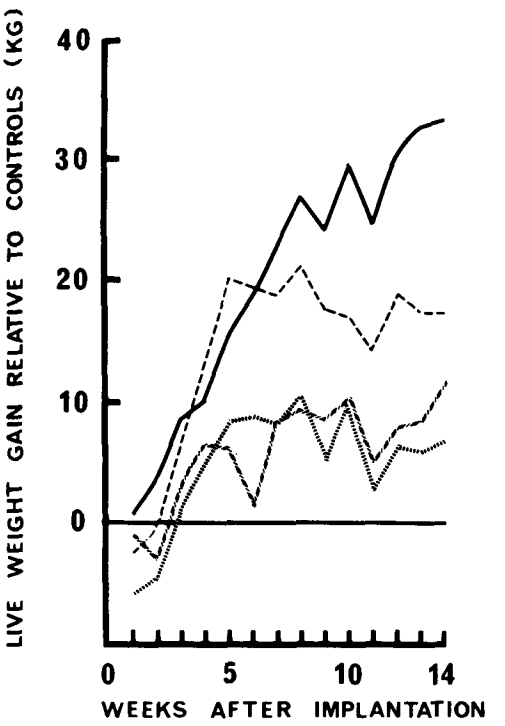

Figure 3. Difference in live weight gains of implanted steers relative to live weight gain of controls. (1) Steers treated with estradiol (IIIII); (2) steers treated with trenbolone acetate (▰▰); (3) steers treated with trenbolone acetate and estradiol placed in separate ears (– – –); (4) steers treated with a mixture of trenbolone acetate and estradiol in one ear (——).

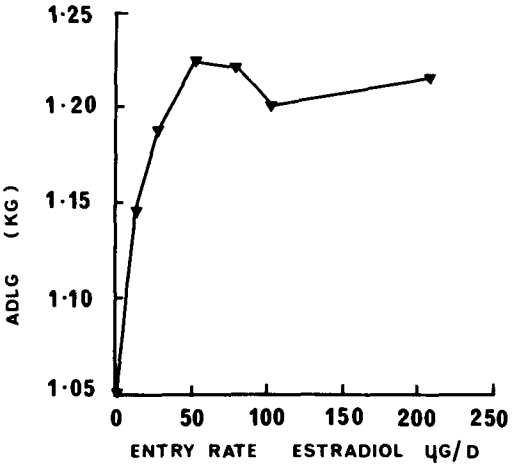

Figure 4. Estradiol entry rate and growth in steers. Each value is the average daily live weight gain (ADLG) by steers given estradiol-impregnated silicone rubber implants with different entry rates of estradiol. The entry rate of estradiol is an average over a 200-d trial period. Data computed from those of Wagner et al. (1979).

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metabolites are either excreted or taken up by the tissues in a nonbiologically active form. There is a long exposure of drug in animals implanted with anabolic agents that may last several months. It can be assumed that a steady state exists between entry rate and metabolic clearance rate, the latter being determined by the rate of metabolism and excretion. Because of the high capacity of the liver and kidney to metabolize or excrete the circulating anabolic agent we can assume that clearance rate equals entry rate. Eighty percent of a 2.8 g dose of trenbolone acetate was cleared into the bile within 24 h after iv administration (Pottier et al., 1981), suggesting that the capacity of the liver to metabolize anabolic agents under field conditions is never exceeded, because most implants contain less than 300 mg of an androgen or 60 mg of an estrogen and these are absorbed over 60 to 150 d.

The half-life of many anabolic agents is measured in minutes rather than hours (Pottier et al., 1975; Harrison, 1981). Consequently anabolic agents must enter the circulation continuously to maintain effective concentrations in the target tissues. Very little is known about the metabolism of the active drug in the target tissues. The anabolic agent enters the target cell and has to form a complex with its receptor before a cellular response is initiated. Whether the agent is metabolized before or after complex formation is not known, but metabolism within the cell will affect the active concentration of the agent in the cell.

The disposition of anabolic agents has been determined by administering radiolabeled agents and measuring their metabolic fate in tissues, fluids and excreta. These radiometric studies have been reported for DES administered orally and parenterally to cattle (Aschbacher et al., 1975; Rumsey et al., 1975a,b), trenbolone acetate in cows (Pottier et al., 1975), estradiol in pigs (Bottoms et al., 1977), estradiol and estradiol benzoate in cattle (Dunn et al., 1977) and progesterone in cattle (Estergreen et al., 1977). All of these studies showed high concentrations of residues at the site of administration and in the bile, feces and urine. Higher concentrations of residues were found in liver and kidney than in other tissues. Further analysis of the nature of the radiolabeled residues indicated that there was considerable metabolism of the anabolic agents either to conjugates or metabolites with altered functional groups or to unidentified metabolites that were irreversibly bound to tissues (Rico, 1983).

Rumsey et al. (1975a) compared the effects of orally administered DES with those of implanted DES and concluded that the pattern of distribution of DES among feces, urine, tissues and bile was similar, suggesting that the DES excretion mechanism functions independently of route of administration. Those authors also concluded that the need for a much larger oral dose than an implanted dose to elicit a similar growth response is probably the result of the efficiency with which the liver metabolizes DES entering through the portal system.

Excretion

Anabolic agents are excreted in feces, urine and milk. Anabolic agents are not allowed in milking animals except in cull cows destined for meat. The liver converts anabolic agents to less biologically active metabolites and conjugates that are usually more water soluble. Those that are secreted in the bile enter the feces and are excreted. Some of the conjugated fraction may be hydrolyzed to the free form of the agent by bacteria in the gastrointestinal tract with the free form (e.g., DES) than excreted or reabsorbed via the enterohepatic circulation. Some of the metabolites formed in the liver may enter the systemic circulation and enter other tissues as residues. The role of the liver is very important in the metabolic fate of anabolic agents, but no studies have been conducted using farm animals.

Anabolic agents are metabolized and excreted from the kidney into the urine. The distribution of excreted radioactivity between urine and feces measured in the radiometric studies was not the same for all anabolic agents or species. For example, the excretion of radioactivity after administration of an implant of ^{3}H-estradiol combined with trenbolone acetate to the veal calf is shown in figure 5. The concentration of residues in feces was higher than in urine, showing that feces was the preferred route of excretion, and there also was little change in the ratio of residues in feces and urine throughout the treatment period (Riis and Suresh, 1976). On the other hand, after oral feeding of ^{14}C-estradiol to swine, 75% of the radioactivity

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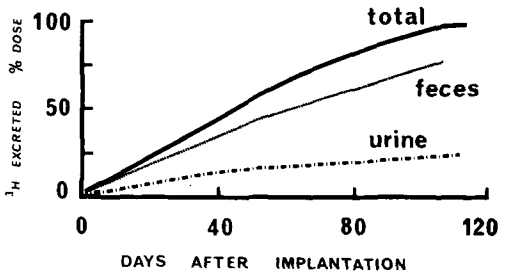

Figure 5. Excretion of radioactivity from calves implanted with ^{3}H-estradiol and trenbolone acetate. Veal calves were implanted with a mixture of 20 mg ^{3}H-estradiol and 140 mg trenbolone acetate. Radioactivity in feces and urine was measured throughout the treatment period and the cumulative total expressed as a percentage of the initial dose. Total radioactivity is the sum of radioactivity in feces and urine. Data from Riis and Suresh (1976).

was excreted in the urine within 72 h and less than 10% was excreted in the feces (Bottoms et al., 1977). When radiolabeled DES was administered to steers and sheep approximately 60 to 70% and 84 to 95%, respectively, of the excreted label appeared in the feces (Aschbacher and Thacker, 1972). These observations indicate the most suitable fluid, tissue or excreta to be used for the measurement of residues of anabolic agents. For example, it is not possible to detect residues of hexestrol in muscle or fat of steers implanted with 36 or 60 mg hexestrol, because the concentrations are less than 50 ppt, which is below the sensitivity of the radioimmunoassay method (Harwood et al., 1981). However, as depicted in figure 6, the presence of hexestrol in feces of similarly implanted steers was easily detectable throughout the treatment period.

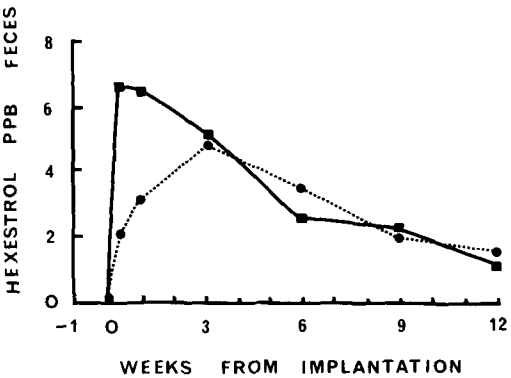

Figure 6. Residues of hexestrol in feces of steers. Each value is the mean for the concentration, measured by radioimmunoassay, of hexestrol in feces of five steers implanted with 60 mg hexestrol (−■−■−) or five steers implanted with 36 mg hexestrol and 300 mg trenbolone acetate (−●−●−). The mean concentration (tissue blank) ± SE in the 10 steers before implantation was .1 ± .1 ng/g feces.

Conclusion

There is rapid metabolism and excretion of anabolic agents with short half-lives in blood, and metabolic clearance rate is equal to entry rate. Therefore, formulation and route of administration, which determine entry rate, are most important in the use of anabolic agents. There is an optimum concentration of anabolic agent in the systemic circulation that results in maximum increase in the growth rate of farm animals. The optimum blood concentration should be maintained throughout the treatment period and is best achieved by slow release implants.

The pattern of exponential absorption from compressed pellets or injections is not ideal and high concentrations and residues are found in the early part of the treatment period.

The high metabolic clearance rate and rapid excretion of anabolic agents influences the distribution of residues. The lowest concentrations are found in muscle and fat, somewhat higher concentrations are present in the liver and kidney and the highest concentrations are in the bile, urine and feces.

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Metabolism of anabolic androgenic steroids

WILHELM SCHÄNZER

Anabolic androgenic steroids (AAS) are misused to a high extent in sports by athletes to improve their physical performance. Sports federations consider the use of these drugs in sports as doping. The misuse of AAS is controlled by detection of the parent AAS (when excreted into urine) and (or) their metabolites in urine of athletes. I present a review of the metabolism of AAS. Testosterone is the principal androgenic steroid and its metabolism is compared with that of AAS. The review is divided into two parts: the general metabolism of AAS, which is separated into phase I and phase II metabolism and includes a systematic discussion of metabolic changes in the steriod molecule according to the regions (A–D rings), and the specific metabolism of AAS, which presents the metabolism of 26 AAS in humans.

INDEXING TERMS: sports medicine • drug assays • gas chromatography–mass spectrometry

The high extent of misuse of synthetic anabolic androgenic steroids (AAS) in sports by athletes to improve their physical performance became public in the 1970s.[1] The International Olympic Committee (IOC) in 1974 banned the use of synthetic AAS by athletes. This prohibition was also adopted by all national and international sports federations. This ban encouraged drug testing laboratories to develop methods for the detection of misused anabolic steroids. The first methods, based on radioimmunoassay techniques *[1, 2]*, failed to take into account the high extent of metabolism of AAS. For detecting and identifying AAS metabolites, gas chromatography–mass spectrometry (GC-MS) is the current method of choice *[3–5]*. Because most AAS are extensively metabolized and the parent steroids are detected for only a short period after administration, the detection of AAS metabolites allows one to confirm the misuse of AAS for a longer time. To follow this strategy, investigators must determine the metabolism of anabolic steroids in humans, elucidate the structures of the metabolites, and, if possible, synthesize the metabolites for use as reference materials. This has been done over the past years for the main metabolites of those AAS that are the most frequently misused *[6]*.

Confirmation of AAS misuse is based on comparison of the electron impact (EI) mass spectrum, or selected ion profile, and GC retention time of the trimethylsilyl (TMS) derivatives of the steroid and (or) its metabolite(s) with the corresponding data obtained from synthesized reference substances *[6]* or from characterized reference substances (e.g., metabolites) isolated from urine in an excretion study.

In 1984 the use of testosterone was also banned by the IOC and by all other sports federations. A method for the detection of administered testosterone was developed by Donike et al. *[7]* in 1983, in which the ratio of urinary excreted testosterone glucuronide to epitestosterone glucuronide was used as an indicator for testosterone administration.

The following discussion on the metabolism of anabolic steroids is based mainly on published results from various working groups and from my own group's published and unpublished investigations. All investigations were performed as urinary excretion studies with male subjects. The studies followed the ethical agreement of the University. All studies in our laboratory were based on self-administration of a single oral dose of an anabolic steroid. The identification of metabolites was confirmed by GC-MS. Several metabolites were synthesized to confirm their structures, and in several cases conformation was based on comparison with structurally related steroids. Characteristic fragment ions of AAS as TMS derivatives in their EI mass spectrum were used to confirm the proposed structures. Chemical reactions and enzymatically catalyzed conversions of functional groups were also used as evidence for their structures.

Testosterone and Synthetic AAS

Testosterone (Fig. 1) is the principal androgenic steroid and is produced in males mainly in the testis. In females, smaller amounts of testosterone are produced by the ovary and the adrenal gland. Testosterone was first discovered in 1935 by David et al. *[8]*, who isolated it from the testis of bulls but did not identify its structure. The structure elucidation by synthesis was performed in the same year independently by Butenandt and Hanisch *[9]* and Ruzicka and Wettstein *[10]*. For this work Butenandt and Ruzicka were awarded the Nobel prize in 1939.

Interest in testosterone, which possesses anabolic and andro-

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[1] Nonstandard abbreviations: AAS, anabolic androgenic steroids; IOC, International Olympic Committee; GC-MS, gas chromatography–mass spectrometry; EI, electron ionization; and TMS, trimethylsilyl.

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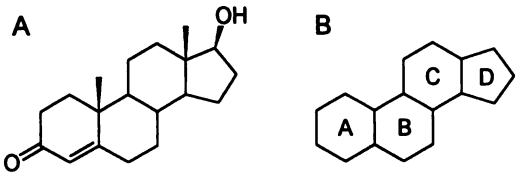

Fig. 1. (*A*) Structural formula of testosterone; (*B*) basic structure of steroids, the perhydrocyclopentanophenanthrene ring system.

genic properties, is based on its ability to stimulate anabolic activities. In medical treatment the use of testosterone improves recovery from catabolic states. Soon after testosterone was identified, it was seen to be not effective when given orally or by parenteral injection, being very rapidly absorbed to the portal blood system and metabolized in the liver. To circumvent this first-pass effect, users administer testosterone as an ester or chemically modified (synthetic AAS). Because of the anabolic and androgenic effects of testosterone, investigators have also wanted to synthesize AAS that have more anabolic and less androgenic activity than testosterone.

The first synthetic anabolic steroids were methyltestosterone, mestanolone, and methandriol, all synthesized by Ruzicka et al. in 1935 *[11]*. In all these steroids, a methyl group is introduced at position C-17α, which makes the 17α-methyl steroids orally effective by slowing their metabolism. The 17α-methyl group is not removed and inactivation occurs only after alteration of the A-ring. The importance of the therapeutic use of AAS in treatment of catabolic conditions was recognized in the 1950s, after which an enormous number of steroids were synthesized and tested for potency. For example, metandienone *[12, 13]* and stanozolol *[14, 15]*, two of the most frequently misused AAS, were synthesized in 1955 and 1959, respectively. Besides 17α-methylation, further modifications were made to reduce the rate of metabolic inactivation. Alteration of the A-ring by introduction of a double bond at C-1,2 yielded metandienone. In stanozolol, a pyrazol ring was condensed to the A-ring, which greatly slowed the rate of metabolic transformation.

The metabolism of testosterone can be discussed as a basic metabolic pathway for all synthetic AAS. The enzymes that convert testosterone to its distinct metabolites are also active towards AAS when similar groups and configurations are present. The metabolism of testosterone has been investigated in various tissues in vivo and in vitro in several animal models and in clinical studies in humans *[16–20]*. Several of these studies were performed with [^{14}C]testosterone to identify possible testosterone metabolites unambiguously. Overviews on the high number of metabolites have been published *[21, 22]*. The main excreted testosterone metabolites 3α-hydroxy-5α-androstan-17-one (androsterone), 3α-hydroxy-5β-androstan-17-one (etiocholanolone), 3β-hydroxy-5α-androstan-17-one (epiandrosterone), 5α-androstane-3α,17β-diol, 5β-androstane-3α,17β-diol, and 5α-androstane-3β,17β-diol are detected in routine urine samples for drug testing and are part of the so-called steroid profiling. These most abundant metabolites are produced by oxidoreductive reactions at C-3, C-4, C-5, and C-17. Hydroxylated metabolites generated by different isoenzymes of cytochrome P-450 are not discussed here because their excretion into human urine is extremely low.

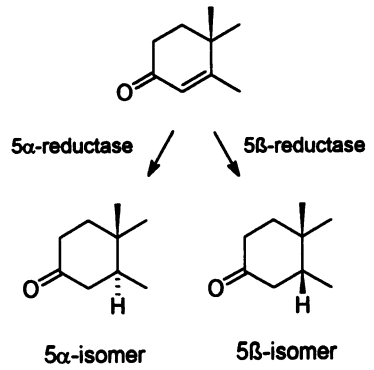

Fig. 2. A-ring metabolism: 5α- and 5β-reduction of 3-keto-4-ene steroids.

General Metabolism of AAS

The following overview is a systematic discussion of metabolic changes in the steroid molecule according to the regions (A–D rings; Fig. 1) of the perhydrocyclopentanophenanthrene ring system, the basic structure of all steroids. These changes are generally grouped into two kinds of metabolism, phase I and phase II, the latter also being referred to as conjugation of the steroid.

Phase I Metabolism

Phase I reactions usually convert the steroid by enzymatically catalyzed reactions (e.g., oxidation, reduction, or hydroxylation) into more polar compounds to inactivate the drug and to facilitate its elimination from the body.

A-RING METABOLISM

5α- and 5β- reduction. The initial and rate-limiting step in the metabolism of 3-keto-4-ene steroids, such as testosterone, is the reduction of the C-4,5 double bond. The reduction yields an asymmetric center at C-5, such that two isomers with 5α- (hydrogen at C-5 below the planar molecule) and 5β-configuration (hydrogen at C-5 above the planar molecule) can be formed (Fig. 2). The enzymes catalyzing the reactions, 5α-reductase and 5β-reductase, are located mainly in the liver *[23]*—5α-reductase primarily in the endoplasmic reticulum and 5β-reductase in the cytoplasm. Both enzymes require NADPH as a cofactor *[24]*. Once the double bond is reduced, the 3-keto group is immediately transformed, as discussed later.

Hydrogenation of the C-4,5 double bond of metandienone and some structurally related A-ring substituents after reduction of the 3-keto group was discussed by Masse et al. *[25]*.

Table 1 summarizes the proposed and observed reduction to 5α- and 5β-isomers for all discussed 3-keto-4-ene AAS.

The extent of 5α- and 5β-isomer produced depends on the structure of the steroid, as Table 2 summarizes for the metabolism of AAS in one male individual. 3-Keto-androsta-1,4-diene structures, such as metandienone and boldenone, do not produce 5α-isomers *[26–28]*. Differences in the D-ring structure also strongly influence the activity of both enzymes. As shown in

Table 1. AAS with a 3-keto-4-en structure, reduction of the C-4,5 double bond in the metabolic pathway.

Anabolic androgenic steroid	5α/5β-Reduced metabolites
Bolasterone	5β-isomer[a]
Boldenone	5β-isomer[a]
Calusterone	5α-/5β-isomer[a]
4-Chloro-1,2-dehydro-17α-methyltestosterone	Detected; only 5β-isomer is proposed
Clostebol	Detected; both isomers are proposed
Fluoxymesterone	Detected; 5α-/5β-isomer
Formebolone	Not detected
Metandienone	5β-isomer[a]
Methyltestosterone	5α-/5β-isomer[a]
Mibolerone	Detected; only 5β-isomer is proposed
Nandrolone	5α-/5β-isomer[a]
Norclostebol	Detected, both isomers are proposed
Norethandrolone	Literature, both isomers are reported
Oxymesterone	Not detected
Testosterone	5α-/5β-isomer[a]
Trenbolone	Not detected

[a] See also Table 2.

Table 2 the metabolism of testosterone to its reduced 5α- and 5β-isomers occurred in a ratio of 1:6, whereas for the 17-keto metabolites (androsterone and etiocholanolone) the ratio was ~1:1. For the reduction of 11β-hydroxyandrost-4-ene-3,17-dione, however, which mostly was metabolized to the 5α-isomer, the 5α/5β ratio was ~15:1.

3α- and 3β-hydroxy- reduction. After reduction of the C-4,5 double bond, which is nonreversible, the 3-keto group in the 5α-isomer is rapidly reduced by either 3α-hydroxysteroid dehydrogenase or 3β-hydroxysteroid dehydrogenase (Fig. 3) *[29]*. In the metabolism of testosterone after oral administration or intramuscular injection *[30]*, mainly 3α-hydroxy isomers are produced and only small amounts of the 3β-hydroxy-5α metabolite are generated. In the metabolism of AAS having a secondary 17β-hydroxy group, 3β-hydroxy-5α-androstan isomers are formed, e.g., for nandrolone (Schänzer and Donike, unpublished data, 1988–93), drostanolone *[31]*, mesterolone *[31, 32]*, and clostebol (Schänzer, Horning, and Donike, unpublished results, 1993). For clostebol we found that the 3β-sulfate was a long-term excreted metabolite. For no 17β-hydroxy-17α-methyl steroid—whether a 5α- or a 5β-isomer—has a 3β-hydroxy metabolite been reported.

3-Keto reduction of a 5β-steroid yielded the 3α-hydroxy structure. The possible formation of the 3β-hydroxy isomer has not been reported, indicating that the reaction does not occur, or that it occurs to such a small extent that it is not detected by the analytical method used (Fig. 3).

1,2-Hydrogenation of 3-keto-androst-1,4-diene steroids. The hydrogenation of the C-1,2 double bond in 3-keto-androsta-1,4-diene steroids (Fig. 4, left) has been reported for metandienone *[26]*. An excretion study with orally applied 17α-methyl-5β-androst-1-ene-3α,17β-diol *[26]* showed that the C-1,2 double bond was reduced in the presence of the allylic 3-hydroxy group, and both steroids were excreted into urine after conjugation.

1,2-Dehydrogenation of 3-keto-4-ene steroids. Endogenous excretion of a 1,2-dehydrogenated steroid (Fig. 4, right) in humans was reported in 1995 *[33]* for androsta-1,4-diene-3,17-dione (boldenone). Some individuals (at least 3 in 10 000 routine doping control samples) excrete small amounts of the main metabolites of boldenone. The origin of endogenously produced

Table 2. Stereospecific metabolism of 3-keto-4-ene steroids to 5α- and 5β-steroids (in relation to the D-ring structure of the metabolite) for one individual.

		D-ring structure			
		17β-Hydroxy		17-Keto	
Substance	Applied amount, mg	5α[a]	5β[a]	5α[a]	5β[a]
d_3-Testosterone	20	13	87	53	47
	2	9	91	47	53
d_7-11β-Hydroxyandrost-4-en-3,17-dione	20	NE	NE	94	6
Nandrolone	20	15	85	72	28
Methyltestosterone	10	17	83	—	—
	100	14	86	—	—
Bolasterone	20	0	100	—	—
Calusterone	40	22	78	—	—
Boldenone	22	0	100	0	100
	80	0	100	0	100
Metandienone	22	0	100	—	—
	40	0	100	—	—

[a] Results expressed as % of steroid in 5α or 5β form.
NE, not estimated; d_3, deuterated at C-16,16,17; d_7, deuterated at C-2,2,3,4,6,6,16,16.
Source: Schänzer and Donike, unpublished data (1988–93).

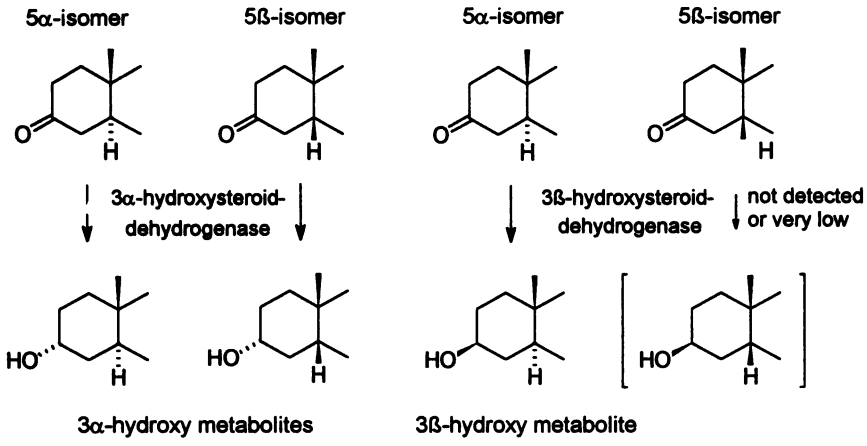

Fig. 3. A-ring metabolism: reduction of 3-keto groups with 3α-hydroxysteroid dehydrogenase and 3β-hydroxysteroid dehydrogenase.

boldenone and its metabolites is unclear. Presumably, unusual bacteria in the gut have 1,2-dehydrogenase activity to convert testosterone, or a precursor, to the 1,2-dehydro steroid. This endogenous production must be considered in drug testing control, with subjects to be followed by endocrinological studies to confirm or rule out the endogenous formation of boldenone and its metabolites. A 1,2-dehydro metabolite is also produced in the metabolism of fluoxymesterone (Schänzer and Horning, unpublished); a 1,2-dehydro metabolite with a 6β-hydroxy structure was identified and its structure elucidated by comparison with a synthesized reference substance. This metabolic reaction occurs to only a small extent (5% of that of the 6β-hydroxylated metabolite). The formation of this 3-keto-androsta-1,4-diene metabolite is possible because the rate of A-ring metabolism in fluoxymesterone is very slow and a high amount of 6β-hydroxyfluoxymesterone with unchanged A-ring structure is present in the body. The formation of this metabolite in the gut seems possible but could be excluded. 1,2-Dehydrogenation is not seen for other 3-keto-androst-4-ene steroids, in which the A-ring is rapidly metabolized as described above.

Further A-ring metabolism of special A-ring modified anabolic steroids. Depending on the type of modification to the A-ring, further metabolites can be identified, as described in detail in the literature for the metabolism of stanozolol (an A-ring condensed pyrazol derivative) *[34]*, oxymetholone *[35, 36]*, formebolone *[37]*, and metenolone *[38]*. See also the specific sections on these compounds presented later.

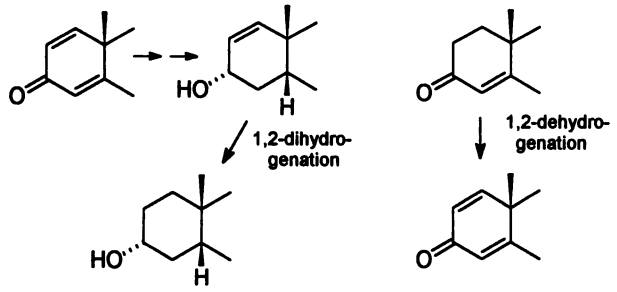

Fig. 4. A-ring metabolism: (*left*) 1,2-hydrogenation of the C-1,2 double bond in 3-keto-androsta-1,4-diene steroids; (*right*) 1,2-dehydrogenation of 3-keto-androst-4-ene steroids to 3-keto-androsta-1,4-diene steroids.

B-RING METABOLISM

6β-Hydroxylation. Metabolism of the B-ring is most pronounced for 17β-hydroxy-17α-methyl steroids where A-ring reduction is hampered by the presence of a C-1,2 double bond, e.g., in metandienone and 4-chlor-1,2- dehydro-17α-methyltestosterone, and by the C-9α fluorine atom in fluoxymesterone. Hydroxylation at position C-6β is the main metabolic route in these anabolic steroids (Fig. 5) *[39–41]*. Excretion studies have not detected any 6α-hydroxy metabolites.

6,7-Dehydrogenation. 6,7-Dehydrogenation is a minor metabolic pathway, observed only in the metabolism of metandienone *[42]*. The generation of this metabolite is proposed to originate from an unknown conjugate. In an excretion study with metandienone, the 6,7-dehydro metabolite was obtained after diethyl ether extraction of the alkalinized (pH >12) urine but not at pH 7.0. This conjugate was isolated from urine via fractionation from an Amberlite resin (XAD-2) (Schänzer and Donike, unpublished data, 1988–93; see also *Conjugation at the B-ring*). When the isolated fractions were alkalinized (pH >12), the 6,7-ene metabolite was obtained. These results can be explained with a basic labile conjugate possibly conjugated at C-6 or C-7, which generates a 6,7-ene when cleaved. A 6,7-dehydro product of testosterone has also been reported in incubation experiments of liver homogenates of rats with testosterone *[43]*.

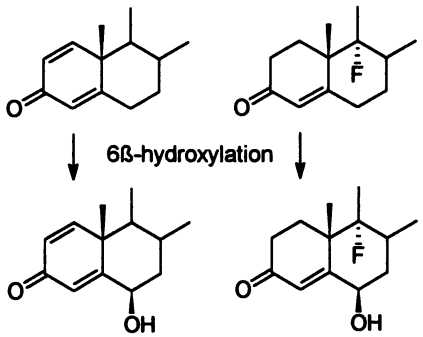

Fig. 5. B-ring metabolism: 6β-hydroxylation.

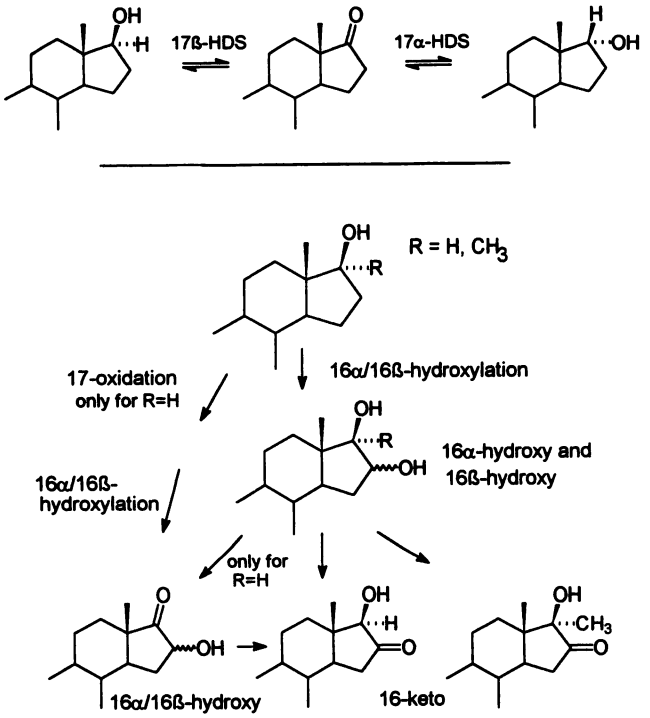

Fig. 6. D-ring metabolism: (*top*) oxidation of 17β-hydroxy groups by 17β-hydroxysteroid dehydrogenase (17β-HDS) and formation of 17α-hydroxy steroids through the reduction of 17-ketones by 17α-hydroxysteroid dehydrogenase (17α-HDS); (*bottom*) 16-hydroxylation and formation of 16-keto metabolites.

C-RING METABOLISM: 12-HYDROXYLATION

Metabolic changes of anabolic steroids at the C-ring are modest. 12-Hydroxylation was first proposed by Dürbeck et al. *[44]* for the metabolism of 4-chlor-1,2-dehydro-17α-methyltestosterone. The proposed 12-hydroxylation of metandienone, stanozolol *[26, 34]*, and 4-chlor-1,2-dehydro-17α-methyltestosterone could be confirmed *[6]*. The TMS derivatives of these metabolites show ion fragments at *m/z* 143 and 170. The ion *m/z* 170 was detected in a synthesized 12-hydroxylated 17β-hydroxy-17α-methyl steroid *[26]*. The ion *m/z* 143 is the typical D-ring fragment of TMS derivatives of 17β-hydroxy-17α-methyl steroids, whereas *m/z* 170 is a C-D-ring fragment and occurs after hydroxylation at C-12. The stereochemistry at C-12 (C-12α or C-12β) is not known.

D-RING METABOLISM

17-Oxidation of the 17β-hydroxy group. The most well-known metabolic pathway of 17β-hydroxy steroids is enzymatic oxidation by 17β-hydroxysteroid dehydrogenase to form the 17-keto steroid (Fig. 6, top) *[45]*. 17-Keto metabolites are the main excreted metabolites of testosterone and all AAS having a secondary 17β-hydroxy group, such as boldenone, clostebol, drostanolone, mesterolone, methenolone, nandrolone, norclostebol, and stenbolone.

17β-Hydroxylation of 17-keto steroids. The 17-keto group can be converted back to the hydroxy group by the same enzyme, 17β-hydroxysteroid dehydrogenase, to give the 17β-hydroxy configuration. The extent of this equilibrium depends on the rate of subsequent metabolic steps (e.g., 17β-conjugation and A-ring reduction). Oral administration of 20 mg of 16,16,17-[$^2H_3$]testosterone to two male volunteers (Schänzer and Donike, unpublished data, 1988–93) was followed by the excretion of 16,16,17-[$^2H_3$]testosterone and 16,16-[$^2H_2$]testosterone in a molar ratio of 2:1. This experiment clearly shows that testosterone is oxidized, and the 17-keto product is, to a considerable extent, reduced back to the 17β-hydroxy steroid.

17α-Hydroxylation of 17-keto steroids. Excretion of a 17α-hydroxy metabolite is observed only in the human metabolism of trenbolone *[46]*. The 17α-hydroxy group is assumed to be formed via the 17-keto metabolite (Fig. 6, top), which in turn supports the probable existence of a 17α-hydroxysteroid dehydrogenase in humans. This assumption is also supported by the excretion of 17-epitestosterone (17α-testosterone). Its production via the 17-keto metabolite has been discussed by Williams *[45]*, but generation of 17α-testosterone (epitestosterone) was not observed after oral administration of testosterone to males *[30]*. The explanation for this can be the location of the 17α-hydroxysteroid dehydrogenase in testosterone-producing organs (testis, ovary, and kidney). In the organs perhaps epitestosterone is formed as a side product in the synthesis of testosterone via androst-4-ene-3,17-dione. An exogenous application of testosterone would be followed by rapid metabolism in the liver to such an extent that unchanged testosterone would scarcely reach the regions where the 17α-hydroxysteroid dehydrogenase enzyme is located.

16α- and 16β-hydroxylation. Hydroxylation at C-16α and C-16β has been reported for several anabolic steroids *[6, 26, 34, 44, 47-49]*. 16-Hydroxylation is well described in the metabolism of estrogens *[50]*. Stereospecific hydroxylations are observed at C-16α and C-16β (Fig. 6, bottom), but the extent of formation of both isomers differs for different AAS. There is no general rule, and in some cases only one isomer is excreted. The exact elucidation of the isomers is not always performed. In the metabolism study of stanozolol *[34]*, the possible four isomers with the 16,17-dihydroxy-17-methyl structure were synthesized, and their GC retention indices (determined as per-TMS derivatives) showed the following order: 16β-hydroxy-17-epistanozolol < 16α-hydroxy-17-epistanozolol < 16α-hydroxy-stanozolol < 16β-hydroxystanozolol. This elution order was also confirmed for further synthesized 16,17-dihydroxy-17-methyl isomers *[26]*.

16-Oxidation. The excretion of 16-keto metabolites of AAS has not been described before. 16-Oxidation was published for estrogens *[50]*. 16-Keto steroids are produced by enzymatic oxidation of the corresponding 16α/16β-hydroxy steroids (see Fig. 6). We recently identified 16-keto steroids in the metabolism of 4-chloro-1,2-dehydro-17α-methyltestosterone *[51]*. This metabolic route of

16-oxidation in humans was also shown in the metabolism of stanozolol in horses.[2]

17-Epimerization. The excretion of 17α-hydroxy-17β-methyl steroids after application of 17β-hydroxy-17α-methyl steroids has been discussed for several years. The formation of the 17-epimers via the sulfoconjugate will be discussed later, in *Phase II Metabolism.*

Further D-ring metabolism. In the metabolism of noreth-androlone, a 17β-hydroxy-17α-ethyl AAS, hydroxylation takes place at the β-position of the 17α-ethyl group *[52]* (see *Noreth-androlone* section). A possible hydroxylation at the 17α-methyl group in 17β-hydroxy-17α-methyl steroids has not yet been identified.

18- (19-) HYDROXYLATION

Evidence has been published for 18-hydroxylation of AAS with a secondary 17β-hydroxy group such as mesterolone, methenolone, and stenbolone *[53]*. Conformation was based on characteristic fragment ions in the EI mass spectrum of the isolated metabolites. The TMS-derivatized primary hydroxy group at C-18 generates a fragment ion *m/z* 103, which is possible only for a primary hydroxy group. It has been difficult to distinguish whether the location of the hydroxy group is at C-19 or C-18. The structural elucidation of Masse and Goudreault *[53]* was based on ion fragments of the unchanged A-ring including the unaltered methyl group at C-19. Horning and I also identified a primary hydroxy group in the metabolism of fluoxymesterone, a 17β-hydroxy-17α-methyl steroid (unpublished data). Experiments to localize the position of the hydroxy group were not able to distinguish exactly between the C-18 and C-19 positions.

Phase II Metabolism

Phase II reactions, which are also named conjugation reactions, act to couple the anabolic steroid or its metabolite with glucuronic acid or sulfate. Conjugation helps elimination of the steroid from the body. Both conjugation reactions are enzymatically controlled, glucuronidation involving UDP-glucuronic acid as a substrate and sulfatation 3′-phosphoadenosine 5′-phosphosulfate.

Not all anabolic steroids and their metabolites are excreted as conjugates. Thus we can distinguish between unconjugated "free" and conjugated metabolites excreted. Unconjugated excreted AAS include oxandrolone, metabolites of oxandrolone, several metabolites of metandienone, fluoxymesterone, 4-chloro-1,2-dehydro-17α-methyltestosterone, formebolone, and two metabolites of stanozolol, in low amounts.

Conjugation of the steroid at the A-ring: sulfatation and glucuronidation of the 3-hydroxy group. In the metabolism of anabolic steroids, reduction of the 3-keto group yields mainly the 3α-hydroxy configuration (see *3α- and 3-β reduction*). The 3α-hydroxy steroids are conjugated with glucuronic acid (Fig. 7) regardless of whether the steroid has a 5α- or 5β-configuration *[54]*. 3β-Hydroxy steroids, on the other hand, are excreted as sulfates *[54]*. 3α-*O*-β-Glucuronides are the major metabolites of AAS; however, some androgens are excreted also as sulfates, e.g., androsterone, etiocholanolone, epiandrosterone (major metabolite excreted), testosterone, and epitestosterone.

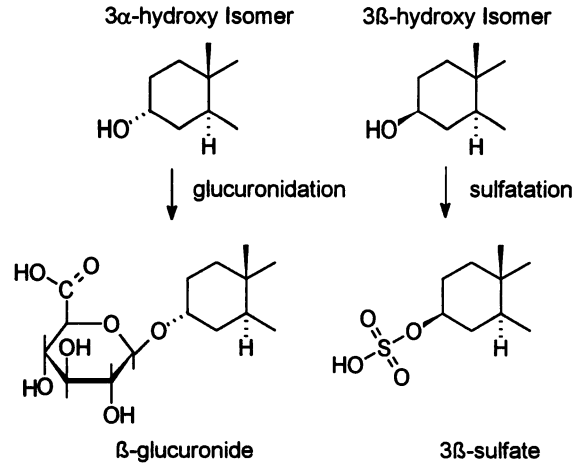

Fig. 7. Conjugation of the 3-hydroxy group: glucuronidation of the 3α-hydroxy group and sulfatation of the 3β-hydroxy group.

Conjugation at the B-ring. Conjugation of steroids at the B-ring has not been published. In the metabolism of metandienone, the 6β-hydroxymetandienone is excreted mainly as a labile conjugate (Schänzer and Donike, unpublished data, 1988–93). The conjugate, which could be isolated from urine by use of HPLC, was stable at pH 3–8 but hydrolyzed spontaneously in alkaline aqueous solution to yield 6β-hydroxymetandienone. The conjugate also hydrolyzed in urine after several days when stored at 4 °C. The nature of this conjugate has not been established yet. Another unknown B-ring conjugate, which is converted to a 6,7-dehydro metabolite of metandienone, was discussed above (see *6,7-Dehydrogenation*).

Conjugation at the D-ring.

1) Glucuronidation of the secondary 17β-hydroxy group. Glucuronidation at the 17β-hydroxy group in secondary 17β-hydroxy steroids (Fig. 8, left) is well known for testosterone. AAS with secondary 17β-hydroxy groups such as methenolone, mesterolone, drostanolone, and clostebol are excreted as conjugates that are readily hydrolyzed with β-glucuronidase from *Escherichia coli*. This enzyme (used throughout, unless otherwise specified) is highly specific for hydrolysis of β-glucuronides of alcoholic groups, especially in steroids. The specificity of this enzymatic hydrolysis allows us to assume that these conjugated steroids are excreted as 17β-glucuronides. Experiments to determine the configuration of these conjugates are in progress.

2) Glucuronidation of the tertiary 17β-hydroxy group in 17β-hydroxy-17α-methyl steroids. Glucuronidation of tertiary 17β-hydroxy groups has not yet been published for 17β-

[2] Houghton E. 16-Oxidation of stanozolol in horses. Presented at the 2nd International Symposium on Hormone and Veterinary Drug Residue Analysis, Oud St-Jan, Bruges, Belgium, 1994.

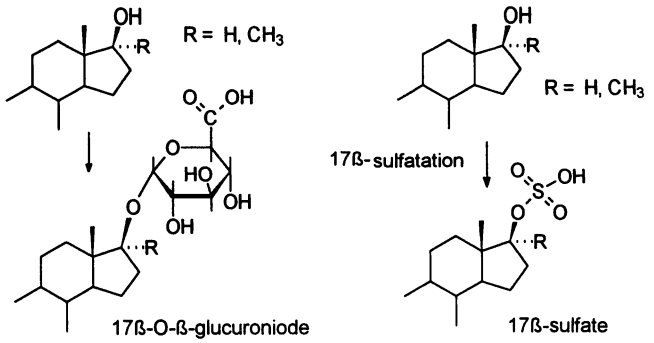

Fig. 8. (*Left*) Glucuronide of secondary and tertiary 17β-hydroxy groups; (*right*) sulfatation of the 17β-hydroxy group.

hydroxy-17α-methyl steroids (see Fig. 8, left, with $R = CH_3$). It has been proposed that the tertiary hydroxy group is sterically hindered for the enzymatic glucuronidation. Surprisingly, 17β-glucuronidation for 17β-hydroxy-17α steroids could be confirmed and was first presented in 1995 (at the 13th Cologne workshop; Schänzer, unpublished). Metandienone is excreted to a small extent as a glucuronide, a finding confirmed by synthesis of metandienone 17β-glucuronide (Schänzer, Horning, Opfermann, unpublished). Fluoxymesterone and 4-chloro-1,2-dehydro-17α-methyltestosterone are also excreted as 17β-glucuronides, but to a much greater extent than metandienone (*ibid.*).

3) Sulfatation of the secondary 17β-hydroxy group. Sulfatation at the 17β-hydroxy group in AAS is possible and is described in the metabolism of testosterone *[55]* (Fig. 8, right). Sanaullah and Bowers discussed detection of epitestosterone and testosterone sulfates in urine by liquid chromatography/MS in 1995 *[56]*.

4) Sulfatation of the tertiary 17β-hydroxy group in 17β-hydroxy-17α-methyl steroids and 17-epimerization. Sulfatation at the 17β-hydroxy group in 17β-hydroxy-17α-methyl steroids was first described for metandienone in horses by Edlund et al. *[57]*. The 17β-sulfate of the tertiary hydroxy group is sterically influenced and decomposes in urine to yield several dehydration products and the corresponding 17-epimeric isomer (17α-hydroxy-17β-hydroxy; Fig. 9). 17-Epimerization has been demonstrated for several 17β-hydroxy-17α-methyl steroids *[26, 58-60]*. The distribution of reaction products has been similar in several studies of AAS excretion.

Studying fluoxymesterone metabolism, Horning and I were able to isolate the assumed sulfate conjugate of fluoxymesterone and to compare it with synthesized fluoxymesterone 17β-sulfate (unpublished). The urinary compound had the same HPLC retention time and ultraviolet absorbance spectrum as the synthesized fluoxymesterone sulfate. When the isolated metabolite and the synthesized product were dissolved in water, they showed the same route of hydrolysis ($t_{1/2}$ ~4 h) and the same distribution of reaction products. From these results we concluded that the precursor of 17-epimeric steroids is the corresponding 17β-sulfate conjugate.

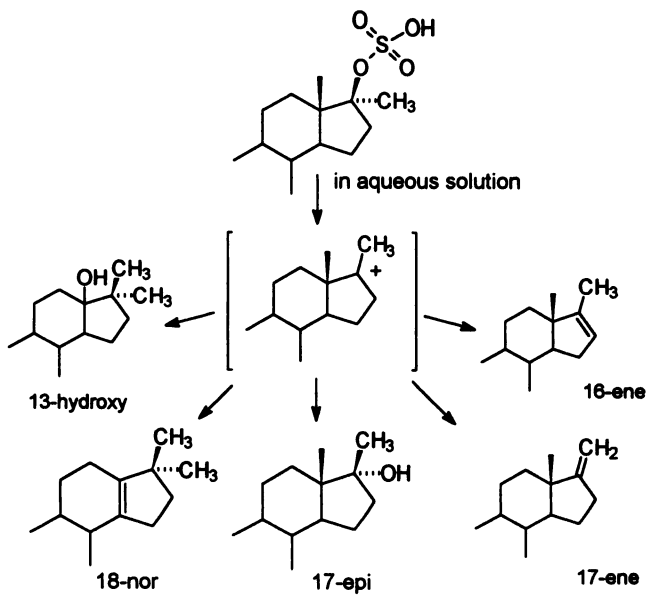

Fig. 9. Degradation of the 17β-sulfate of tertiary 17β-hydroxy groups, with rearrangement to 18-nor-17,17-dimethyl-13-ene, 16-ene, 17-ene, 13-hydroxy-17,17-dimethyl and 17-epimeric steroids.

Metabolism of Specific Anabolic Steroids

BOLASTERONE

The synthesis of bolasterone (7α,17α-dimethyl-17β-hydroxyandrost-4-en-3-one; Fig. 10) was reported in 1959 by Campbell and Babcock *[61]*. There being no published data on the metabolism of bolasterone, Donike and I performed two excretion studies to confirm the main metabolites *[6]*. After oral administration of 20 mg of bolasterone to two male volunteers, excretion of the steroids shown in Fig. 10 was confirmed. Bolasterone was detected in the conjugate fraction and it could be hydrolyzed with β-glucuronidase; thus, we assumed that bolasterone was excreted as a 3-enol glucuronide. This assumption is based on the specificity of the highly purified β-glucuronidase. Given the recent identification of 17β-glucuronides of 17β-hydroxy-17α-methyl steroids, a possible 17β-glucuronide of bolasterone must now also be considered. Confirmation of the structure of this glucuronide is in progress.

Two tetrahydro metabolites—7α,17α-dimethyl-5β-androstane-3α,17β-diol (**2**) and 7α,17β-dimethyl-5β-androstane-3α,17α-diol (**4**)—were excreted as conjugates (hydrolyzed with β-glucuronidase) and could be detected for a longer time after ingestion than the parent steroid. The structure of both metabolites was confirmed by synthesis of the authentic standards *[6, 58]*. The generation of the 17-epimer can be explained via a 17β-sulfate (**3**) (see point 4 in *Phase II Metabolism*). The tetrahydro metabolites are excreted as bis-conjugates, with glucuronidation at the 3α-hydroxy group and sulfatation at the 17β-hydroxy group. The 17β-sulfate undergoes degradation in urine to generate the 17-epimer and the corresponding 18-nor metabolite (**5**). Both reaction products are still conjugated at the 3α-hydroxy group and can be hydrolyzed with β-glucuronidase.

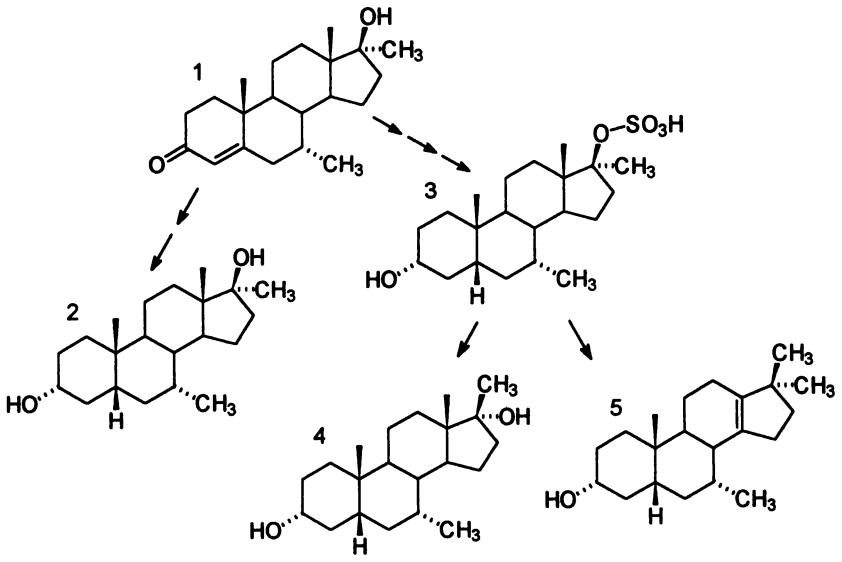

Fig. 10. Metabolism of bolasterone (**1**) to 7α,17α-dimethyl-5β-androstane-3α,17β-diol (**2**); 7α,17α-dimethyl-5β-androstane-3α,17β-diol 17β-sulfate (**3**); 7α,17β-dimethyl-5β-androstane-3α,17α-diol (**4**; 17-epi of **2**); and 7α,17,17-trimethyl-5β-androst-13-en-3α-ol (**5**).

BOLDENONE

Boldenone was synthesized in 1956 by Meystre et al. *[13]*. Its metabolism was investigated by Galletti and Gardi *[27]* in 1971. Donike and I in 1992 published a GC-MS method for screening and identification of boldenone metabolites excreted in human urine *[28]*. The main metabolic routes in boldenone metabolism are shown in Fig. 11. Boldenone itself is excreted as a 17β-conjugate, presumably as a 17β-glucuronide because of its specific hydrolysis with β-glucuronidase. The reduction of the C-4,5-double bond is stereospecific and yields the 5β-configuration. No 5α-metabolite is detected. The main metabolites of boldenone are 17β-hydroxy-5β-androst-1-en-3-one (**2**), 5β-androst-1-ene-3α,17β-diol (**4**), and 3α-hydroxy-5β-androst-1-en-17-one (**6**). 6β-Hydroxylated metabolites are also excreted but only to a low extent *[28]*. Excretion of boldenone and its metabolites in low concentrations in urine has been reported without administration of external steroid *[33]* (see *1,2-Dehydrogenation of 3-keto-4-ene steroids*).

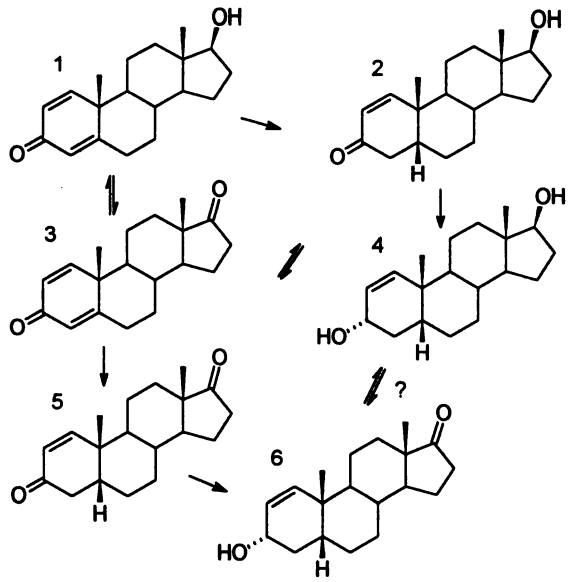

Fig. 11. Metabolism of boldenone (**1**) to 17β-hydroxy-5β-androst-1-en-3-one (**2**); androsta-1,4-diene-3,17-dione (**3**; intermediate, not excreted into urine); 5β-androst-1-ene-3α,17β-diol (**4**); 5β-androst-1-ene-3,17-dione (**5**; intermediate, not excreted into urine); and 3α-hydroxy-5β-androst-1-en-17-one (**6**).

CALUSTERONE

Calusterone (7β,17α-dimethyl-17β-hydroxyandrost-4-en-3-one; Fig. 12), first synthesized by Campbell and Babcock in 1959 *[61]*, is the C-7 epimer of bolasterone (see above). Having found no excretion study with calusterone in the literature, Donike and I performed a preliminary study with oral administration of 40 mg of calusterone to a male volunteer; calusterone itself was excreted unchanged but as a conjugate (unpublished data). Given the hydrolysis of the conjugate with β-glucuronidase, we assume glucuronidation at the tertiary 17β-hydroxy group. Compared with bolasterone, the reduction of the C-4,5 double bond yielded not only the 5β-metabolite (7β,17α-dimethyl-5β-androstane-3α,17β-diol; **2**), but also a 5α-metabolite (7β,17α-dimethyl-5α-androstane-3α,17β-diol; **3** in Fig. 12). The ratio of

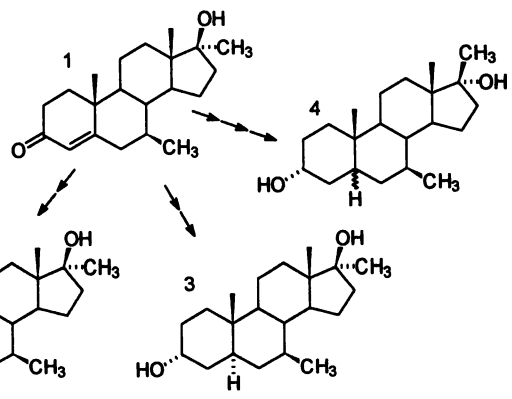

Fig. 12. Metabolism of calusterone (**1**) to 7β,17α-dimethyl-5β-androstane-3α,17β-diol (**2**); 7β,17α-dimethyl-5α-androstane-3α,17β-diol (**3**); and **4**, the 17-epimeric steroid of **2** or **3**.

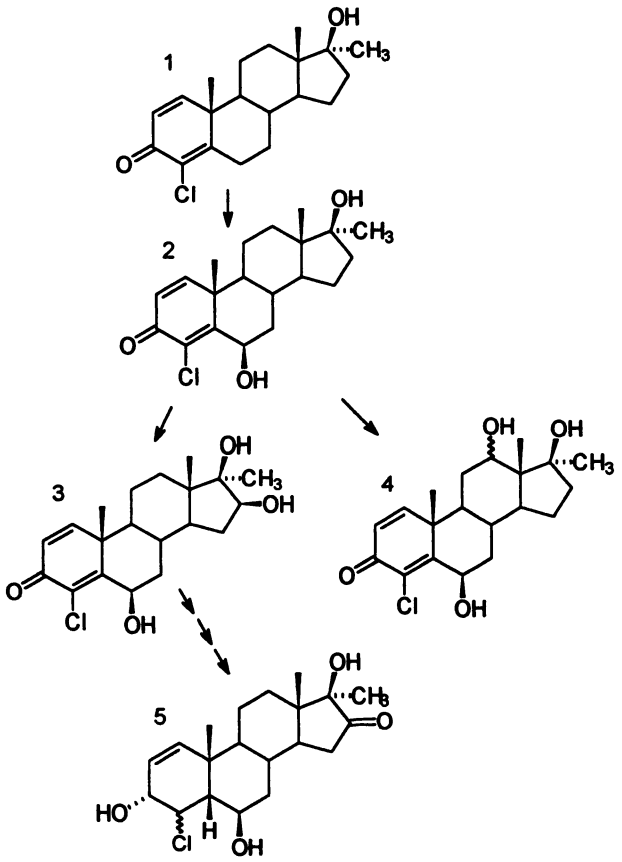

Fig. 13. Metabolism of 4-chloro-1,2-dehydro-17α-methyltestosterone (**1**) to 6β-hydroxy-**1** (**2**); 6β,16β-dihydroxy-**1** (**3**); 6β,12ξ-dihydroxy-**1** (**4**); and the long-term excreted metabolite 4ξ-chloro-3α,6β,17β-trihydroxy-17α-methyl-5β-androst-1-ene-16-one (**5**).

the 5β- to 5α-metabolite was ~4:1 (Table 2). We also identified excretion of a 17-epimeric tetrahydro metabolite, **4** (fully A-ring reduced), present at ~10% of the concentration of the 5β-tetrahydro metabolite.

4-CHLORO-1,2-DEHYDRO-17α-METHYLTESTOSTERONE

The anabolic steroid 4-chloro-1,2-dehydro-17α-methyltestosterone (Fig. 13) was first synthesized in 1960 by Schubert et al. *[62]*. Marketed by Jenapharm in East Germany, it was the steroid most misused by athletes in East Germany (DDR) through 1990. In metabolic studies of 4-chloro-1,2-dehydro-17α-methyltestosterone in humans, Schubert et al. *[48, 49]* reported the urinary excretion of the parent steroid, a 6β-hydroxy (**2**), a 16β-hydroxy, and a 6β,16β-dihydroxy metabolite (**3**). In 1983 Dürbeck et al. *[44]* studied the metabolism of 4-chloro-1,2-dehydro-17α-methyltestosterone by GC-MS. They confirmed the formation of the 6β-hydroxy and 6β,16β-dihydroxy metabolite but did not detect the parent drug and the 16β-hydroxy metabolite. Another metabolite present in substantial quantities was detected for which the proposed structure was a 6β,12-dihydroxy metabolite (see above, *12-Hydroxylation*). Currently, the misuse of 4-chloro-1,2-dehydro-17α-methyltestosterone is controlled by monitoring the 6β-hydroxy metabolite (**2**), which has been synthesized and made available as a reference substance *[6, 41]*. Two additional metabolites identified from excretion studies, the 6β,16β-dihydroxy (**3**) and 6β,12-dihydroxy *[4]* forms, are also used to screen for 4-chloro-1,2-dehydro-17α-methyltestosterone in urine samples of athletes *[6]*.

A recent reinvestigation of the metabolism of 4-chloro-1,2-dehydro-17α-methyltestosterone has found that several metabolites are excreted for a long time after administration *[51]*. One long-term metabolite, 4-chloro-3α,6β,17β-trihydroxy-17α-methyl-5β-androst-1-en-16-one (**5**) (Fig. 13), could be detected for >9 days after oral administration of 40 mg of 4-chloro-1,2-dehydro-17α-methyltestosterone.

CLOSTEBOL (4-CHLOROTESTOSTERONE)

Clostebol (4-chloro-17β-hydroxyandrost-4-en-3-one) was synthesized in 1956 by Camerino et al. *[63]*, and Ringold et al. *[64]*. The first metabolism studies were published by Starka et al. *[65]* in 1969 and by Castegnaro and Sala *[66]* in 1973. The predominant excreted metabolites were oxidized 17-keto products, and the A-ring was reduced to bis and tetrahydro metabolites. The exact configuration of the A-ring-reduced metabolites was not confirmed. After oral administration of 20 mg of clostebol acetate, five main metabolites were detected (Schänzer, Horning, and Donike, unpublished results, 1993) (Fig. 14). The main metabolite excreted is 4-chloro-3α-hydroxyandrost-4-en-17-one (**2**), which has been synthesized as a reference material *[6]*. Two further 17-oxidized and fully A-ring-reduced metabolites (**4** and **5**) are assumed to be the corresponding C-4-chlorinated analogs of androsterone and etiocholanolone. A further abundant hydroxy metabolite (**7**), hydroxylated at C-16 and fully A-ring-reduced, was detected and confirmed by GC-MS. The configuration at C-16 and of the A-ring is still unknown.

Besides these metabolites, which are all excreted as glucuronides, a 17-keto tetrahydro metabolite has been isolated as a sulfate conjugate. This metabolite has the most prolonged detection time after administration of clostebol. Because the A-ring is fully reduced and the metabolite is excreted as a sulfate, we propose the configuration 4ξ-chloro-3β-hydroxy-5α-androstan-17-one (**6**). The configuration at C-4 (bearing the chloride ion) is still unclear. The exact clarification of the A-ring configuration, including the 4-chloro configuration (α or β), for all tetrahydro products is of interest because it will yield information about the reduction mechanisms of the 5α and 5β reductases.

DROSTANOLONE

Drostanolone (17β-hydroxy-2α-methyl-5α-androstan-3-one; Fig. 15) was first synthesized in 1959 by Ringold et al. *[67]*. It is taken orally as the 17-propionic acid ester. Its metabolism was investigated by GC-MS and reported by DeBoer et al. in 1992 *[31]*, who confirmed that the parent steroid was excreted as a conjugate that can be hydrolyzed with β-glucuronidase. The main metabolite is the 17-keto-oxidized 3α-hydroxy-reduced product 3α-hydroxy-2α-methyl-5α-androstan-17-on (**4**), which, in comparison with testosterone metabolism, is the 2α-methyl androsterone analog. This metabolite has been synthesized as a reference substance *[6]*. Possible reaction pathways for genera-

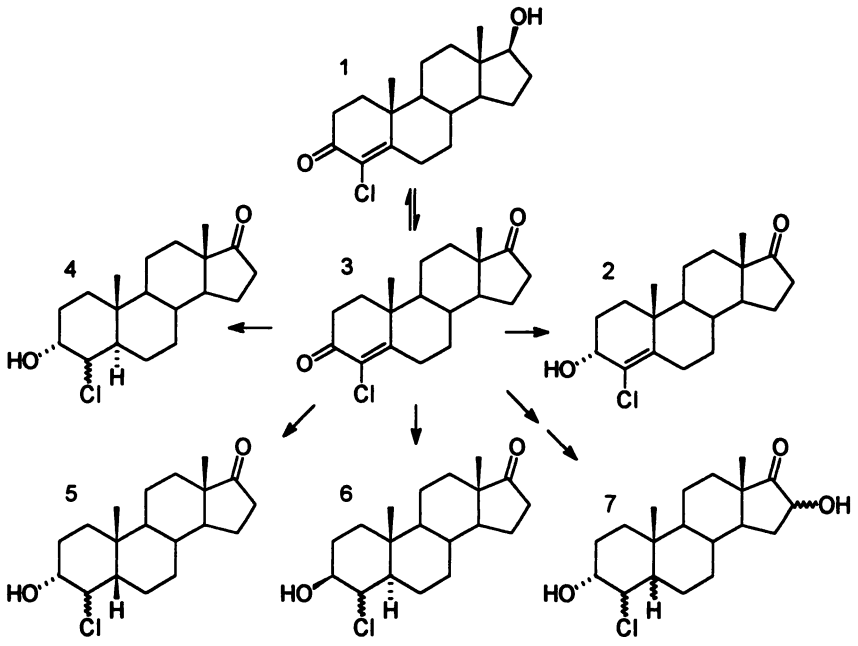

Fig. 14. Metabolism of clostebol (**1**) to 4-chloro-3α-hydroxyandrost-4-en-17-one (**2**); 4-chloroandrost-4-ene-3,17-dione (**3**; intermediate, not excreted into urine); and other metabolites.

Proposed structures for metabolites **4–7**: **4**, 4ξ-chloro-3α-hydroxy-5α-androstan-17-one; **5**, 4ξ-chloro-3α-hydroxy-5β-androstan-17-one; **6**, 4ξ-chloro-3β-hydroxy-5α-androstan-17-one; **7**, 4ξ-chloro-3α,16ξ-dihydroxy-5ξ-androstan-17-one.

tion of this metabolite are shown in Fig. 15. A minor metabolite, 2α-methyl-5α-androstane-3α,17β-diol (**3**), is also formed.

ETHYLESTRENOL

Ethylestrenol (19-nor-17α-pregn-4-en-17-ol) was synthesized by De Winter et al. in 1959 *[68]*. Metabolism of ethylestrenol in humans was reported by Ward et al. in 1977 *[69]*, who found that ethylestrenol metabolism was similar to that of noretheandrolone (see below). Metabolism of ethylestrenol proceeds through hydroxylation at C-3 (Fig. 16). The main metabolites 17α-ethyl-5α-estrane-3α,17β-diol (**2**), 17α-ethyl-5β-estrane-3α,17β-diol (**3**; synthesis described elsewhere *[6]*), and 17α-ethyl-5ξ-estrane-3α,17β,21-triol (**4**) have been confirmed. The 3α-hydroxy configuration of the metabolites is proposed because all metabolites are excreted as conjugates that can be hydrolyzed with β-glucuronidase. Further hydroxy metabolites have been detected, but their structures remain unknown. Recently, Geyer, Donike, and I confirmed the excretion of 3α-hydroxy-5α-estran-17-one (norandrosterone) and 3α-hydroxy-5β-estran-17-one (noretiocholanolone) in low amounts as metabolites (unpublished), both of which are the main metabolites in the metabolism of nandrolone (19-nortestosterone) (see *Nandrolone* section).

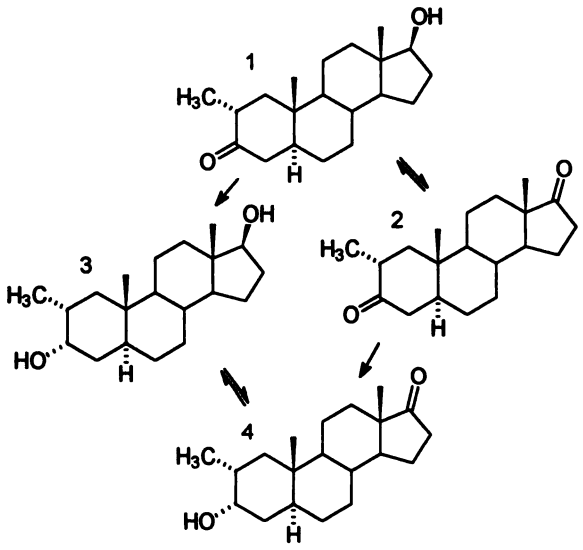

Fig. 15. Metabolism of drostanolone (**1**) to 2α-methyl-5α-androstane-3,17-dione (**2**; intermediate, not excreted into urine); 2α-methyl-5α-androstane-3α,17β-diol (**3**); and 3α-hydroxy-2α-methyl-5α-androstan-17-one (**4**).

FLUOXYMESTERONE

Fluoxymesterone (9-fluoro-11β,17β-dihydroxy-17α-methylandrost-4-en-3-one) was first synthesized in 1956 by Herr et al. *[70]*. Its metabolism was investigated by Kammerer et al. *[71]*, who excluded the excretion of A-ring-reduced metabolites by comparison with reference substances. Recently, Horning and I prepared an excretion study with 20 and 40 mg of orally applied fluoxymesterone and confirmed the excretion of 20 metabolites after separation by HPLC (unpublished). The main metabolites are shown in Fig. 17. Fluoxymesterone itself is excreted mostly as a 17β-glucuronide and 17β-sulfate. The main metabolites detected are 6β-hydroxy-fluoxymesterone (**2**), 9-fluoro-17α-methylandrost-4-ene-3α,6β,11β,17β-tetrol (**3**), and 9-fluoro-18-nor-17,17-dimethyl-11β-hydroxyandrosta-4,13-diene-3-one (**5**). A little 17-epifluoxymesterone (**6**) was produced, formed by epimerization of the fluoxymesterone 17β-sulfate.

Compared with other 17-epimerization processes of sulfated 17β-hydroxy-17α-methyl anabolic steroids, where the 18-nor steroid and the epimeric steroid were formed in equal amounts, the amount of 18-nor product produced was ~5 times that of the 17-epimer of fluoxymesterone sulfate. This difference seems

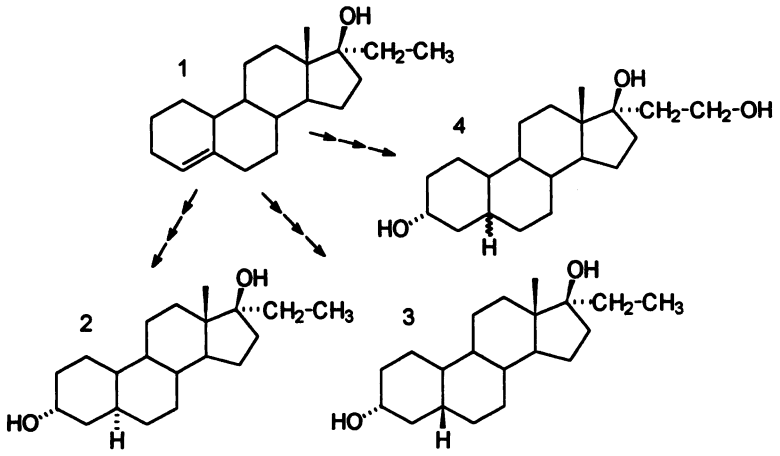

Fig. 16. Metabolism of ethylestrenol (**1**) to 17α-ethyl-5α-estrane-3α,17β-diol (**2**); 17α-ethyl-5β-estrane-3α,17β-diol (**3**); and 17α-ethyl-5ξ-estrane-3α,17β,21-triol (**4**).

to be due to the presence of the 11β-hydroxy group in fluoxymesterone, which favors the rearrangement process that generates the 18-nor steroid. In contrast to the results of Kammerer et al. *[71]*, we identified fully A-ring-reduced metabolites: two tetrahydro fluoxymesterone metabolites and two 6β-hydroxy tetrahydro metabolites. No evidence of a 11-keto metabolite was found.

FORMEBOLONE

Formebolone (2-formyl-11α,17β-dihydroxy-17α-methylandrosta-1,4-diene-3-one; Fig. 18, left) was synthesized in 1965 by Canonica et al. *[72]*. GC-MS investigation of formebolone metabolism in humans was published by Masse et al. *[37]* in 1991, who proposed metabolite structures as related to their EI mass spectra. To confirm the structure of the primary metabolite, Donike and I performed an excretion study with 20 mg of formobolone taken orally *[6]*. A reduced metabolite, 2-hydroxymethyl-11α,17β-dihydroxy-17α-methylandrosta-1,4-diene-3-one (**2**; Fig. 18, left), was found in the unconjugated urine fraction after basic extraction and was identified by comparison with the synthesized compound *[6]*.

FURAZABOL

Furazabol (17β-hydroxy-17α-methyl-5α-androstano[2,3-*c*]-furazan; Fig. 18, right) was synthesized in 1965 by Ohta et al. *[73]*. Metabolic studies in rats were published by Takegoshi et al. *[74]*. A GC-MS investigation of furazabol metabolism in humans *[75]* showed that furazabol was excreted as a conjugate that could be hydrolyzed with β-glucuronidase. From this result it is assumed that furazabol is excreted as a 17β-glucuronide. Further work to confirm this assumption is in progress. A 16-hydroxy metabolite was also identified, although its exact configuration (16α-hydroxy or 16β-hydroxy) is still not determined. 16-Hydroxy metabolites of 17-hydroxy-17-methyl steroids can be easily confirmed by GC-MS from the characteristic EI mass spectra for D-ring fragment ions from the TMS derivative *[26, 34]*.

MESTANOLONE

Mestanolone (17β-hydroxy-17α-methyl-5α-androstan-3-one) was first synthesized in 1935 by Ruzicka et al. *[11]*. Masse et al. *[25]* published in 1991 a GC-MS investigation of the mestanolone metabolic pathway (Fig. 19). After oral administration to a male volunteer, 17α-methyl-5α-androstane-3α,17β-diol (**2**)

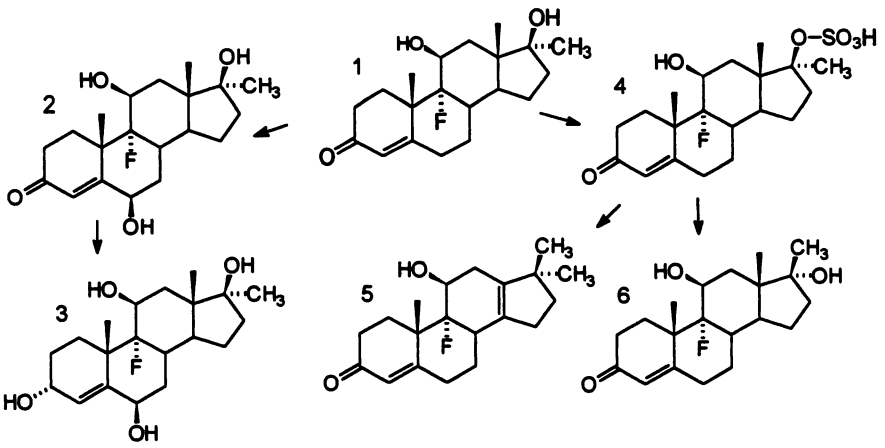

Fig. 17. Metabolism of fluoxymesterone (**1**) to 6β-hydroxyfluoxymesterone (**2**); 9-fluoro-17α-methylandrost-4-ene-3α,6β,11β,17β-tetrol (**3**); fluoxymesterone 17β-sulfate (**4**); 9-fluoro-11β-hydroxy-18-nor-17,17-dimethylandrosta-4,13-dien-3-one (**5**); and 17-epifluoxymesterone (**6**).

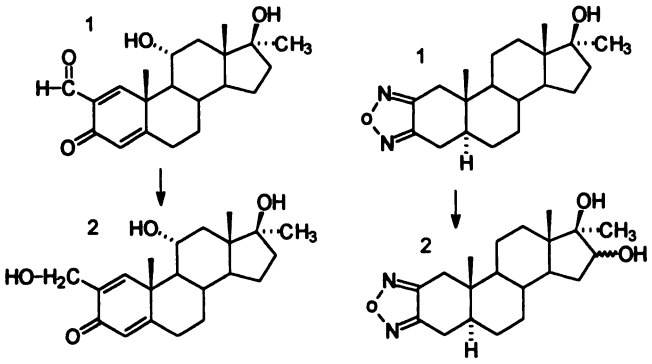

Fig. 18. (*Left*) Metabolism of formebolone (**1**) to its main metabolite 2-hydroxymethyl-11α,17β-dihydroxy-17α-methylandrosta-1,4-dien-3-one (**2**); (*right*) metabolism of furazabol (**1**) to its main metabolite 16ξ-hydroxyfurazabol (**2**).

was excreted as a main conjugate, which can be hydrolyzed with β-glucuronidase. Mestanolone is rapidly reduced by 3α-hydroxysteroid dehydrogenase. This result is in agreement with the metabolism of methyltestosterone, in which, as an intermediate after 5α-reduction, mestanolone is produced and is further reduced to the corresponding 3α-hydroxy-5α-androstane product. No parent drug nor 3β-hydroxy metabolites were observed.

Recent experiments (Schänzer and Horning, unpublished) have shown that initially a 17-epimer [17β-methyl-5α-androstane-3α,17α-diol (**3**)] and 18-nor-17,17-dimethyl-5α-androst-13-en-3α-ol (**4**) are excreted in low amounts (1–2% of the main metabolite), both as conjugates (3α-hydroxy glucuronides). This excretion pattern changes after several days. After 14 days, for example, all metabolites were excreted in comparable low amounts. Even later, after as long as 21 days, the excretion of the epimer exceeds the excretion of the main metabolite. The formation of the epimer follows the epimerization process already described via a 17β-sulfate, and the excretion of the 18-nor-17,17-dimethyl product is in agreement with this mechanism. Because all these metabolites have a 3α-hydroxy structure, they are conjugated with glucuronic acid before excretion into urine. The excreted sulfate is therefore a bis-conjugate and,

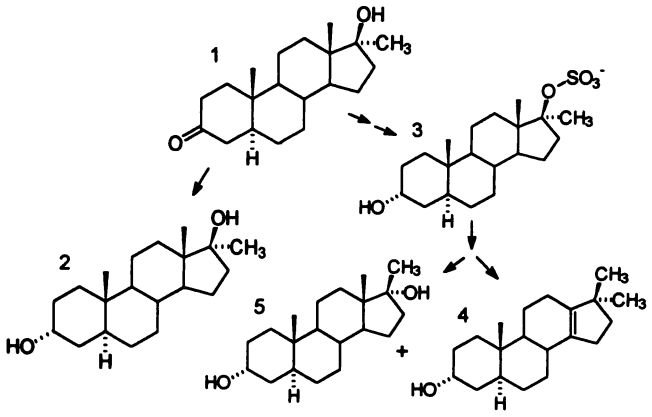

Fig. 19. Metabolism of mestanolone (**1**) to 17α-methyl-5α-androstane-3α,17β-diol (**2**); 17α-methyl-5α-androstane-3α,17β-diol 17β-sulfate (**3**); 18-nor-17,17-dimethyl-5α-androst-13-en-3α-ol (**4**); and 17β-methyl-5α-androstane-3α,17α-diol (**5**).

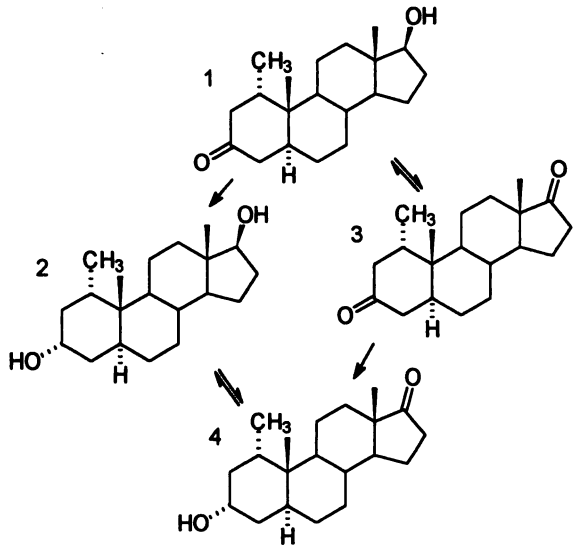

Fig. 20. Metabolism of mesterolone (**1**) to 1α-methyl-5α-androstane-3α,17β-diol (**2**); 1α-methyl-5α-androstane-3,17-dione (**3**; not excreted into urine); and 3α-hydroxy-1α-methyl-5α-androstan-17-one (**4**).

after the rearrangement process, the formed products are still conjugated (3α-hydroxy conjugate).

MESTEROLONE

Mesterolone (17β-hydroxy-1α-methyl-5α-androstan-3-one) was synthesized by Wiechert in 1965 *[76]*. Human metabolism of mesterolone was reported by DeBoer et al. *[31]* and Goudreault and Ayotte *[32]*. Masse and Goudreault reported 18-hydroxylation as a minor metabolic pathway of mesterolone *[53]*. DeBoer confirmed by GC-MS the urinary excretion of the parent steroid (conjugate, hydrolysis with β-glucuronidase), 1α-methyl-5α-androstane-3α,17β-diol (**2**) and the androsterone analog (**4**) as major metabolites (Fig. 20). This metabolite was further elucidated by synthesis *[6]* as 3α-hydroxy-1α-methyl-5α-androstan-17-one (**4**).

METANDIENONE

Metandienone (17β-hydroxy-17α-methylandrosta-1,4-dien-3-one; Fig. 21) was first synthesized in 1955 by Vischer et al. *[12]* by microbiological dehydrogenation of methyltestosterone. In 1956 Meystre et al. *[13]* published a dehydrogenation synthesis of methyltestosterone with selenium dioxide. As the main metabolite of this steroid, 6β-hydroxymetandienone (**2**) was identified in 1963 by Rongone and Segaloff *[39]*. This metabolite was excreted unconjugated. As discussed above (*Conjugation at the B-ring*), recent experiments have shown that 6β-hydroxymetandienone is mainly excreted as a labile conjugate, the structure of which is unknown. A further metabolite, 17-epimetandienone (**5**), was identified and synthesized in 1971 by Macdonald et al. *[77]*. As reported later *[58, 60]*, the 17-epimeric product results from degradation and rearrangement of an excreted 17β-sulfate. Further publications *[42, 44]* present results from GC-MS investigations of the unconjugated fraction.

In 1991, we reported identification of the conjugated A-ring-

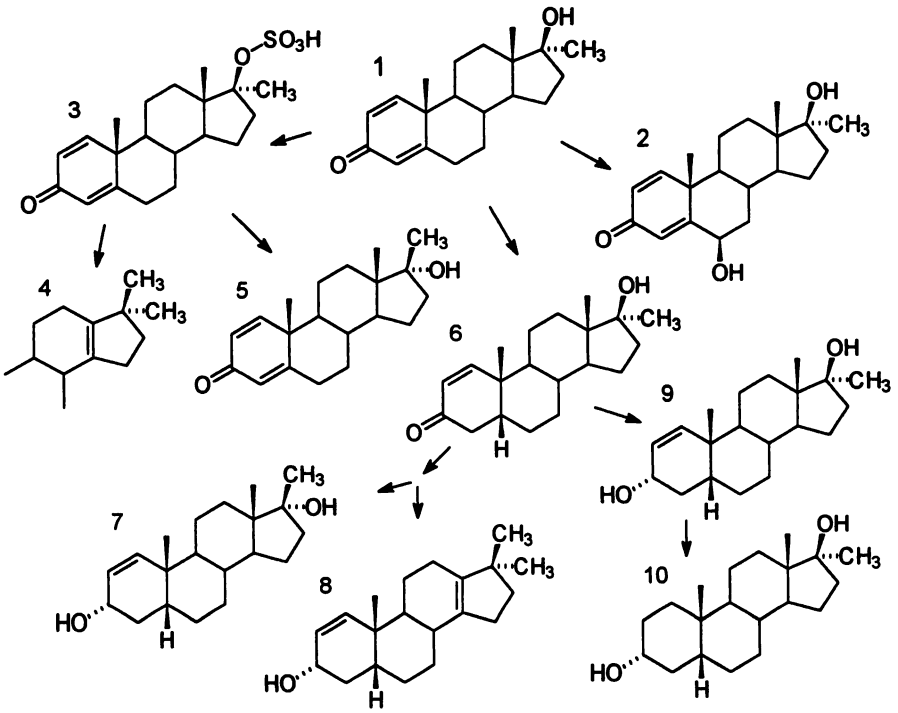

Fig. 21. Metabolism of metandienone (**1**) to 6β-hydroxymetandienone (**2**); metandienone 17β-sulfate (**3**); 18-nor-17,17-dimethylandrosta-1,4,13-trien-3-one (**4**); 17-epimetandienone (**5**); 17β-hydroxy-17α-methyl-5β-androst-1-en-3-one (**6**); 17β-methyl-5β-androst-1-ene-3α,17α-diol (**7**); 18-nor-17,17-dimethyl-5β-androsta-1,13-dien-3α-ol (**8**); 17α-methyl-5β-androst-1-ene-3α,17β-diol (**9**); and 17α-methyl-5β-androstane-3α,17β-diol (**10**).

reduced metabolites *[26]*. These include (Fig. 21): 17β-methyl-5β-androst-1-ene-3α,17α-diol (**7**), 17α-methyl-5β-androst-1-ene-3α,17β-diol (**9**), and 17α-methyl-5β-androstane-3α,17β-diol (**10**). The 17-epimer, **7**, is a long-term excreted metabolite; it can be detected for a very long time after the administration of metandienone. Because the formation of this 17-epimer is followed by the general degradation process to the corresponding 17β-sulfate, it is also possible to detect the 18-nor product, 18-nor-17,17-dimethyl-5β-androsta-1,13-dien-3α-ol (**8**).

METHENOLONE

Methenolone (17β-hydroxy-1-methyl-5α-androstan-3-one; Fig. 22) was synthesized in 1960 by Wiechert and Kaspar *[78]*. Methenolone is applied as an acetate or enanthate ester, the latter being suitable for intramuscular injection. The metabolism of methenolone in humans was investigated by Gerhards et al. in 1965 *[79]*, who identified 3α-hydroxy-1-methylen-5α-androstane-17-one (**4**) as a major metabolite. In this metabolic pathway, the oxidation of the 17β-hydroxy group and reduction of the 3-keto group are in agreement with the metabolism of testosterone to androsterone. Interestingly, the C-1,2 double bond is isomerized to an exocyclic double bond (1-methylene group) by a mechanism still not clarified. Also, much of the parent drug is excreted unchanged into urine. Both the parent drug and **4** are excreted as conjugates that can be hydrolyzed with β-glucuronidase. In 1990 Goudreault and Masse *[38]* published a GC-MS investigation of methenolone metabolism in humans, describing and characterizing several metabolites by their EI mass spectra.

METHANDRIOL

Methandriol (17α-methylandrost-5-en-3β,17β-diol; Fig. 23, left) was synthesized in 1935 by Ruzicka et al. *[11]*. To confirm the main metabolites of methandriol in humans in dope analysis, we investigated the urinary excretion pattern after oral administration of 30 mg of methandriol dipropionate and 20 mg of

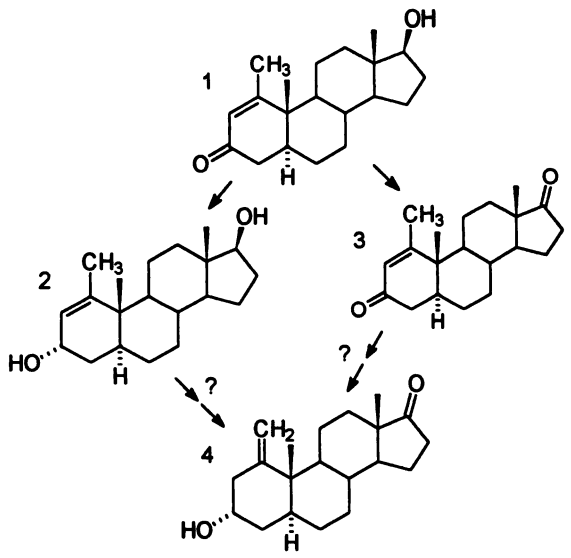

Fig. 22. Metabolism of methenolone (**1**) to 1-methyl-5α-androst-1-ene-3α,17β-diol (**2**); 1-methyl-5α-androst-1-ene-3,17-dione (**3**; intermediate, not excreted into urine); and 3α-hydroxy-1-methylen-5α-androstan-17-one (**4**).

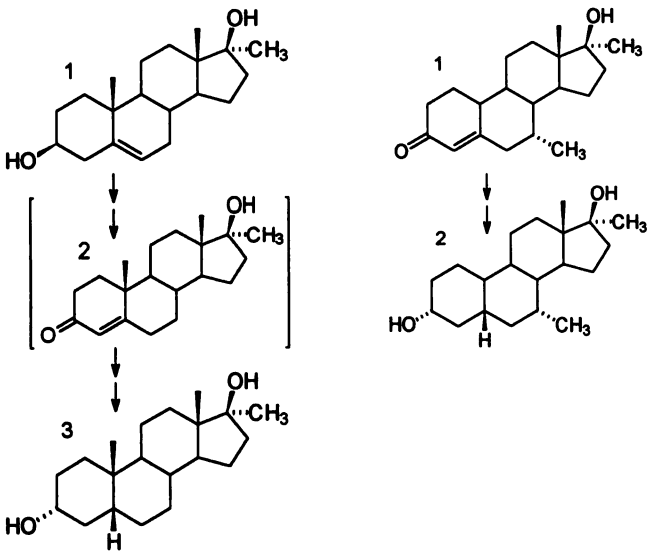

Fig. 23. (*Left*) Metabolism of methandriol (**1**) to methyltestosterone (**2**; proposed intermediate, not excreted into urine) and 17α-methyl-5β-androstane-3α,17β-diol (**3**); (*right*) metabolism of mibolerone (**1**) into its proposed main metabolite 7α,17α-dimethyl-5β-estrane-3α,17β-diol (**2**).

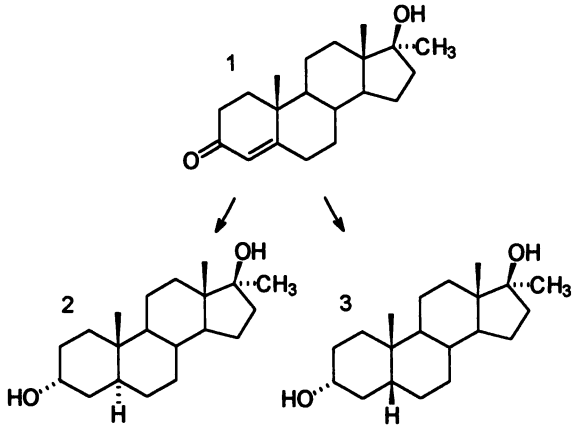

Fig. 24. Metabolism of methyltestosterone (**1**) into its main excreted metabolites 17α-methyl-5α-androstane-3α,17β-diol (**2**) and 17α-methyl-5β-androstane-3α,17β-diol (**3**).

methandriol *[6]*. The parent steroid was excreted as a sulfate, possibly as a 3β-sulfate, in low amounts. Hydrolysis of this metabolite was not possible with the β-glucuronidase from *E. coli* but required an arylsulfatase enzyme from *Helix pomatia*. 17α-Methyl-5β-androstane-3α,17β-diol (**3**; Fig. 23, left) was confirmed as the main metabolite. This metabolite is also a metabolite in the metabolism of metandienone and methyltestosterone; its formation can be explained via an oxidation of the 3β-hydroxy group in methandriol and enzymatic isomerization (steroid-Δ-isomerase) of the C-5,6 double bond to C-4,5. The intermediate so formed (methyltestosterone) is then metabolized mainly to the 5β-isomer. The total amount of excreted metabolites is very low (<5% of the administered steroid); further investigations should be initiated to account for the remaining metabolic products and to elucidate the extent of absorbed substance.

METHYLTESTOSTERONE

Methyltestosterone (17β-hydroxy-17α-methylandrost-4-en-3-one; Fig. 24) was synthesized in 1935 by Ruzicka et al. *[11]*. The metabolism of methyltestosterone in humans was investigated in 1962 by Rongone and Segaloff *[80]*, who identified 17α-methyl-5α-androstane-3α,17β-diol (**2**) and 17α-methyl-5β-androstane-3α,17β-diol (**3**) as the main metabolites. These are the classical metabolites as described for the A-ring metabolism of testosterone. Investigations of 17-epimeric metabolites show that the corresponding 17-epimeric steroids are produced but in very low amounts (2–3.5% of the excreted 17β-hydroxy-17α-methyl metabolites). The ratio of the main metabolic isomers 5α/5β was about 0.17 (Table 2) for the individual investigated.

MIBOLERONE

Mibolerone (17β-hydroxy-7α,17α-dimethylestr-4-en-3-one) was synthesized in 1963 by Segaloff *[81]*. The metabolism of mibolerone was investigated by Bowers *[82]* and after oral application of 20 mg to a male volunteer (Geyer, Schänzer, Donike, unpublished). A major excreted product was the fully A-ring-reduced metabolite, which was excreted as a conjugate. The structure of this metabolite is assumed to be 7α,17α-dimethyl-5β-estrane-3α,17β-diol (**2**; Fig. 23, right); however, this has not yet been confirmed. The proposed structure is in agreement with the reduction of mibolerone in methanol/potassium hydroxide by hydrogen, with platinum dioxide as a catalyst; the reduction yields only one intermediate with 3-keto structure. The 5β-configuration is based on hydrogenation results for the structurally related steroid bolasterone (Fig. 10), which yields only a 5β-product under the same conditions. The 3α-hydroxy structure is in agreement with the hydrolysis of the conjugate (glucuronide) with β-glucuronidase.

NANDROLONE (19-NORTESTOSTERONE)

Nandrolone (17β-hydroxyestr-4-en-3-one; Fig. 25) was synthesized in 1950 by Birch *[83]* and by Wilds and Nelson *[84]* in 1953. The metabolism was investigated by Engel et al. in 1958 *[85]*. The metabolism strongly follows that of testosterone, and the main metabolites have been confirmed as 3α-hydroxy-5α-estran-17-one (**3**, norandrosterone) and 3α-hydroxy-5β-estran-17-one (**2**, noretiocholanolone). The structure of both metabolites was elucidated by synthesis in 1960 *[86]*. Besides these metabolites, a 3β-hydroxy isomer, 3β-hydroxy-5α-estran-17-one (**4**), is also excreted into urine as a 3β-sulfate in an amount similar to that of the 3α-hydroxy metabolites (Schänzer and Donike, unpublished data, 1988–93).

NORCLOSTEBOL

Norclostebol (4-chloro-17β-hydroxyestr-4-en-3-one; Fig. 26) was first synthesized by Camerino et al. *[63, 87]*. We investigated its metabolism after a single oral dose of 20 mg of norclostebol acetate to a male volunteer (Geyer, Donike, Schänzer, unpublished). Only the glucuronide fraction (hydrolyzable

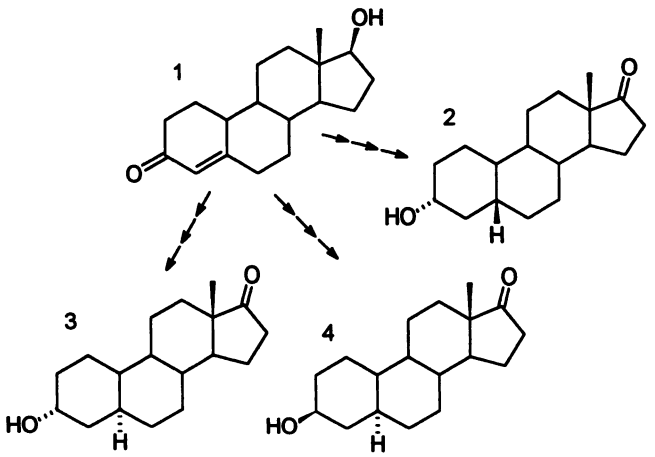

Fig. 25. Metabolism of nandrolone (**1**) to 3α-hydroxy-5β-estran-17-one (**2**), 3α-hydroxy-5α-estran-17-one (**3**), and 3β-hydroxy-5α-estran-17-one (**4**).

with β-glucuronidase) has been analyzed. The four metabolites we detected are in agreement with the metabolism of clostebol (see above). All metabolites have a 17-keto group; two (**4**, **5**) are fully A-ring-reduced metabolites (possible androsterone and etiocholanolone analogs); one has a 3-hydroxy-4-ene structure (**2**); and the fourth is proposed to be C-16 hydroxylated (**6**). The proposed structures presented in Fig. 26 are based on GC-MS analysis of the per-TMS-derivatized steroids.

NORETHANDROLONE

Norethandrolone (17β-hydroxy-17α-ethylestr-4-en-3-one) was synthesized in 1957 by Colton et al. *[88]*. Brooks et al. *[52]* investigated in 1971 the metabolism of norethandrolone in humans and reported three metabolites: 17α-ethyl-5α-estrane-3α,17β-diol (**2**), 17α-ethyl-5β-estrane-3α,17β-diol (**3**), and a tetrahydro metabolite hydroxylated at the ethyl side chain (**4**) (Fig. 27). These studies were confirmed in 1977 by Ward et al. *[69]*. The EI mass spectrum *[6]* of the TMS derivative of 17α-ethyl-5β-estrane-3α,17β, 21-triol (**4**) shows abundant D-ring fragment ions at *m/z* 144 and 157, confirming the hydroxylated position.

OXANDROLONE

Oxandrolone (17β-hydroxy-17α-methyl-2-oxa-5α-androstan-3-one; Fig. 28) was synthesized in 1962 by Pappo and Jung *[89]*. In 1989 Masse et al. *[47]* published a GC-MS investigation of oxandrolone metabolism in men. Oxandrolone is excreted unchanged and metabolized to its 17-epimer (**4**), which has already been explained as a urinary rearrangement product of an excreted 17β-sulfate. In this degradation process the 18-nor-17,17-dimethyl product (**5**) is also formed. Excretion of further 16-hydroxylated metabolites (**2**) of oxandrolone have been confirmed, but in low concentrations. All metabolites including the parent steroid are excreted unconjugated.

OXYMESTERONE

Oxymesterone (4,17β-dihydroxy-17α-methyandrost-4-en-3-one; Fig. 29, left) was synthesized in 1965 by Camerino *[90]*. Little metabolized, it is excreted mainly unchanged *[58]* as a conjugate that can be hydrolyzed with β-glucuronidase. This indicates that oxymesterone is excreted as a 17β-glucuronide.

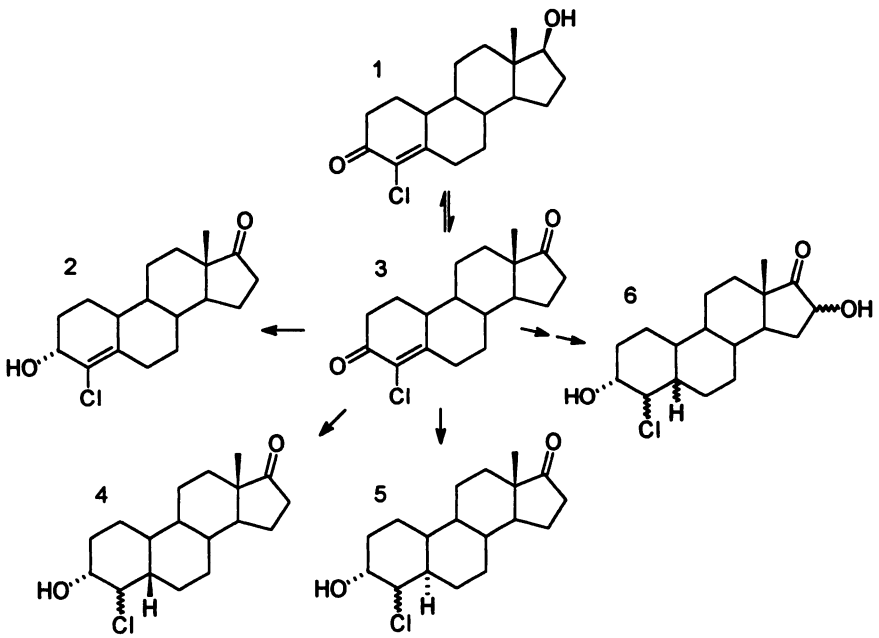

Fig. 26. Metabolism of norclostebol (**1**) to 4-chloro-3α-hydroxyestr-4-en-17-one (**2**); 4-chloroestr-4-ene-3,17-dione (**3**; intermediate, not excreted into urine); and other metabolites.

Proposed structures for metabolites **4–6**: **4**, 4ξ-chloro-3α-hydroxy-5β-estran-17-one; **5**, 4ξ-chloro-3α-hydroxy-5α-estran-17-one; **6**, 4ξ-chloro-3α,16ξ-dihydroxy-5ξ-androstan-17-one.

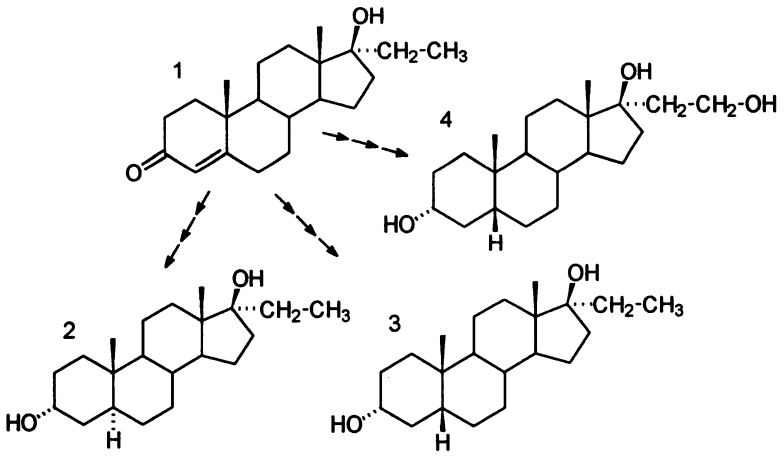

Fig. 27. Metabolism of norethandrolone (**1**) to 17α-ethyl-5α-estrane-3α,17β-diol (**2**); 17α-ethyl-5β-estrane-3α,17β-diol (**3**); and proposed metabolite **4**: 17α-ethyl-5β-estrane-3α,17β,21-triol.

OXYMETHOLONE

Oxymetholone (17β-hydroxy-2-hydroxymethylene-17α-methyl-5α-androstan-3-one) was synthesized in 1959 by Ringold et al. *[67]*. Its metabolism in humans was discussed by Bi et al. *[35, 36]*, who identified the urinary excretion of acidic metabolites (Fig. 30). They reported a 2-carbonic acidic metabolite (**3**) and, further, several seco diacetics as metabolites. In the neutral and basic fractions, several hydroxylated and reduced metabolites were detected, but their structural elucidation was not possible. A known metabolite 17α-methyl-5α-androstane-3α,17β-diol (**4**), which is also a metabolite of methyltestosterone, has been detected. This metabolite originates via oxidation of the 2-hydroxymethylene group to a β-ketocarbonic acid intermediate (**2**), which is then decarboxylated to 17β-hydroxy-17α-methyl-5α-androstan-3-one (not shown in Fig. 30). This product is subsequently reduced to the 3α-hydroxy metabolite (**4**).

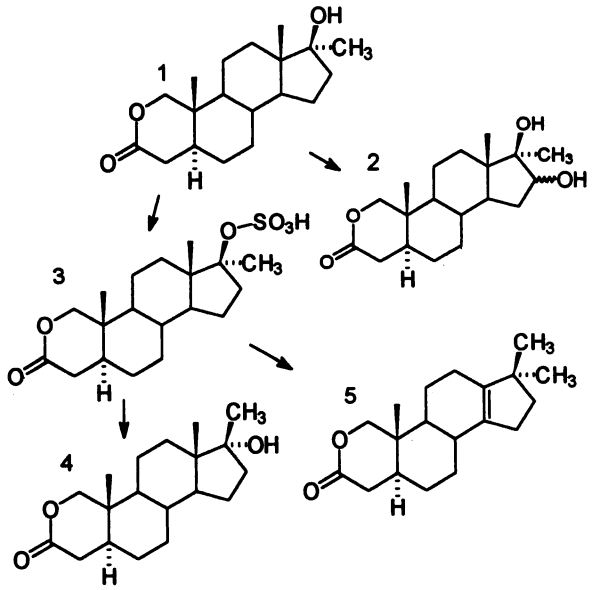

Fig. 28. Metabolism of oxandrolone (**1**) to 16ξ-hydroxyoxandrolone (**2**), oxandrolone 17β-sulfate (**3**), 17-epioxandrolone (**4**), and 18-nor-17,17-dimethyloxandrolone (**5**).

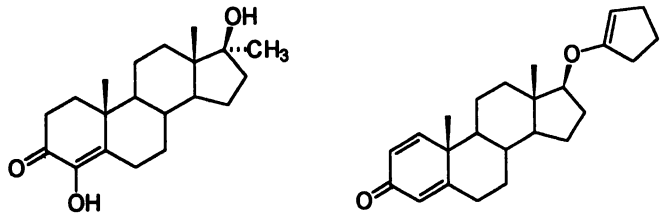

Fig. 29. Structural formulas of oxymesterone (*left*) and quinbolone (*right*).

QUINBOLONE

Quinbolone [17β-(1-cyclopenten-1-yloxy)-androsta-1,4-dien-3-one; Fig. 29, right] is the 17β-cyclopentene ether of boldenone and follows the metabolism of boldenone (see above).

STANOZOLOL

Stanozolol (17β-hydroxy-17α-methyl-5α-androst-2-eno[3,2-*c*]pyrazole; Fig. 31) was synthesized in 1959 by Clinton et al. *[14, 15]*. Stanozolol is synthesized starting with oxymetholone (see Fig. 30), which is condensed with hydrazine to form a pyrazole ring. In 1989 Masse et al. published a GC-MS method

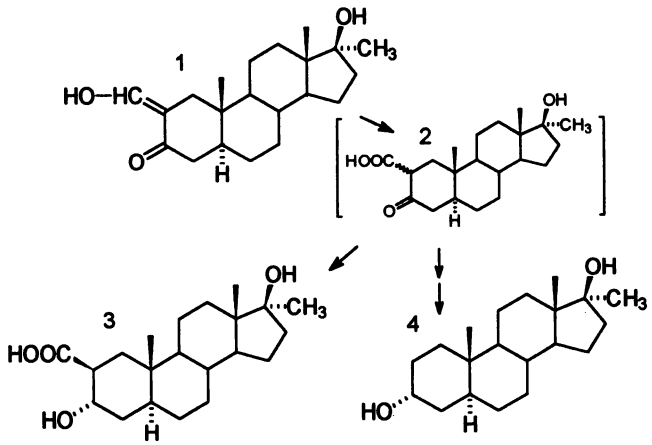

Fig. 30. Metabolism of oxymetholone (**1**) to 17β-hydroxy-3-keto-17α-methyl-5α-androstane-2ξ-carboxylic acid (**2**; intermediate, not excreted into urine); 3α,17β-dihydroxy-17α-methyl-5α-androstane-2β-carboxylic acid (**3**); and 17α-methyl-5α-androstane-3α,17β-diol (**4**).

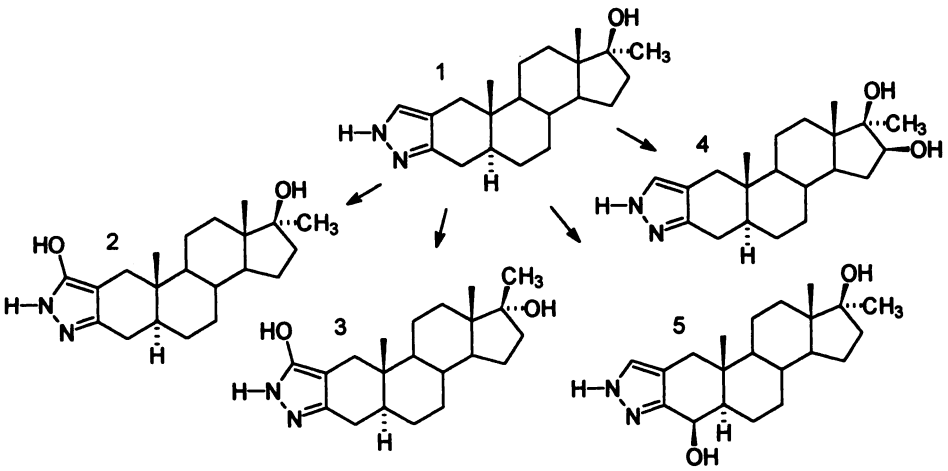

Fig. 31. Metabolism of stanozolol (**1**) to 3′-hydroxystanozolol (**2**), 3′-hydroxy-17-epistanozolol (**3**), 16β-hydroxystanozolol (**4**), and 4β-hydroxystanozolol (**5**).

for identification of stanozolol metabolites *[91]* obtained from urine after oral administration of stanozolol.

The metabolism of stanozolol in humans was extensively investigated by Schänzer et al. *[34]* in 1990. Eleven metabolites were confirmed by GC-MS after separation of the urine extract by HPLC. The main excreted metabolites (Fig. 31) were synthesized: 3′-hydroxystanozolol (**2**), 3′-hydroxy-17-epistanozolol (**3**) *[58]*, 4β-hydroxystanozolol (**5**), and 16β-hydroxystanozolol (**4**). 3′-Hydroxy-17-epistanozolol (**3**) was detected only in the unconjugated fraction (only 1–2% of the quantity of the conjugated excreted metabolites), and the origin of this metabolite can be assumed to be a 17β-sulfate that rearranges in the urine to form the 17-epimer and several other dehydration products (point 4 in *Phase II Metabolism*). The most abundant metabolites—3′hydroxy-, 4β-hydroxy-, and 16β-hydroxystanozolol—were all excreted as conjugates that can be hydrolyzed with β-glucuronidase.

STENBOLONE

Stenbolone (17β-hydroxy 2α-methyl-5α-androst-1-en-3-one) was synthesized in 1960 by Mauli et al. *[92]* and in 1962 by Counsell et al. *[93]*. In 1991 Goudreault and Masse *[94]* published a GC-MS method for identification of urinary excreted metabolites of stenbolone in humans. They identified excretion of unchanged stenbolone, several 17-keto metabolites, and 16-hydroxy metabolites (Fig. 32) and proposed the expected structures.

TRENBOLONE

Trenbolone (17β-hydroxyestra-4,9,11-trien-3-one; Fig. 33), used as a veterinary product, was synthesized in 1963 by Velluz et al. *[95]*. A GC-MS method for detection of trenbolone and its main metabolite 17-epitrenbolone was first presented in 1989[3] and was published in 1991 by DeBoer et al. *[46]*. Trenbolone and 17-epitrenbolone are both excreted as conjugates that can be hydrolyzed with β-glucuronidase. From the specific hydrolysis, one can assume that the liberated trenbolone and its 17-epimer are excreted as β-glucuronides. It also seems possible that trenbolone and its 17-epimer are additionally excreted as sulfates. This has not yet been confirmed and will be an interesting subject for future research.

Conformation of the 17-epimer was based on its GC retention time and the EI mass spectra of different derivatives, all of which show an intense cleavage of the 17α-ether (M^+ − HO-R) or ester group (M^+ − HO-CO-R′). Analysis of trenbolone and its 17-epimer by GC-MS can be problematic when derivatized

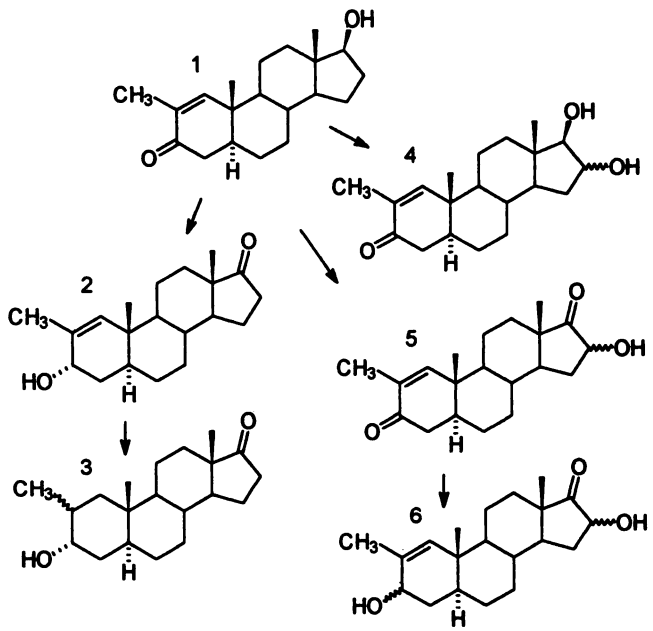

Fig. 32. Metabolism of stenbolone (**1**) to 3α-hydroxy-2-methyl-5α-androst-1-en-17-one (**2**); 3α-hydroxy-2ξ-methyl-5α-androstan-17-one (**3**); 16ξ-hydroxy-stenbolone (**4**); 16ξ-hydroxy-2-methyl-5α-androst-1-ene-3,17-dione (**5**); and 3ξ,16ξ-dihydroxy-2-methyl-5α-androst-1-en-17-one (**6**).

[3] By myself (in June) at the 7th Cologne Workshop in Dope Analysis, and by H. Geyer (in October) at the 4th International Symposium in Doping Control, Moscow.

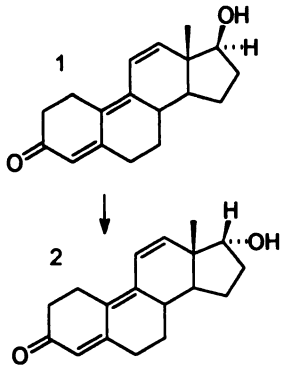

Fig. 33. Metabolism of trenbolone (**1**) to its main metabolite 17-epitrenbolone (**2**).

with MSTFA/TMIS [*N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide/trimethyliodosilane]. Enolization of the 3-keto group yields a highly reactive compound that can yield several decomposition products under GC analysis. In this case, different derivatives are used.

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THE EFFECTS OF SUPRAPHYSIOLOGIC DOSES OF TESTOSTERONE ON MUSCLE SIZE AND STRENGTH IN NORMAL MEN

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ABSTRACT

Background Athletes often take androgenic steroids in an attempt to increase their strength. The efficacy of these substances for this purpose is unsubstantiated, however.

Methods We randomly assigned 43 normal men to one of four groups: placebo with no exercise, testosterone with no exercise, placebo plus exercise, and testosterone plus exercise. The men received injections of 600 mg of testosterone enanthate or placebo weekly for 10 weeks. The men in the exercise groups performed standardized weight-lifting exercises three times weekly. Before and after the treatment period, fat-free mass was determined by underwater weighing, muscle size was measured by magnetic resonance imaging, and the strength of the arms and legs was assessed by bench-press and squatting exercises, respectively.

Results Among the men in the no-exercise groups, those given testosterone had greater increases than those given placebo in muscle size in their arms (mean [±SE] change in triceps area, 424±104 vs. −81±109 mm²; $P<0.05$) and legs (change in quadriceps area, 607±123 vs. −131±111 mm²; $P<0.05$) and greater increases in strength in the bench-press (9±4 vs. −1±1 kg, $P<0.05$) and squatting exercises (16±4 vs. 3±1 kg, $P<0.05$). The men assigned to testosterone and exercise had greater increases in fat-free mass (6.1±0.6 kg) and muscle size (triceps area, 501±104 mm²; quadriceps area, 1174±91 mm²) than those assigned to either no-exercise group, and greater increases in muscle strength (bench-press strength, 22±2 kg; squatting-exercise capacity, 38±4 kg) than either no-exercise group. Neither mood nor behavior was altered in any group.

Conclusions Supraphysiologic doses of testosterone, especially when combined with strength training, increase fat-free mass and muscle size and strength in normal men. (N Engl J Med 1996;335:1-7.)

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ANABOLIC–ANDROGENIC steroids are widely abused by athletes and recreational bodybuilders because of the perception that these substances increase muscle mass and strength,[1-9] but this premise is unsubstantiated. Testosterone replacement increases nitrogen retention and fat-free mass in castrated animals and hypogonadal men,[10-15] but whether supraphysiologic doses of testosterone or other anabolic–androgenic steroids augment muscle mass and strength in normal men is unknown.[1-9] Studies of the effects of such steroids on muscle strength have been inconclusive,[16-33] and several reviews have emphasized the shortcomings of the studies.[1-5,8-10] Some of the studies were not randomized; most did not control for intake of energy and protein; the exercise stimulus was often not standardized; and some studies included competitive athletes whose motivation to win may have kept them from complying with a standardized regimen of diet and exercise.

We sought to determine whether supraphysiologic doses of testosterone, administered alone or in conjunction with a standardized program of strength-training exercise, increase fat-free mass and muscle size and strength in normal men. To overcome the pitfalls of previous studies, the intake of energy and protein and the exercise stimulus were standardized. Because some previous studies had demonstrated significant increases in muscle strength and hyper-

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trophy in experienced athletes but not in sedentary subjects, we studied men who had weight-lifting experience.

METHODS

Study Design

This study was approved by the institutional review boards of the Harbor–UCLA Research and Education Institute and the Charles R. Drew University of Medicine and Science. All the study subjects gave informed written consent. The subjects were normal men weighing 90 to 115 percent of their ideal body weights; they were 19 to 40 years of age and had experience with weight lifting. They were recruited through advertisements in local newspapers and community colleges. None had participated in competitive sports in the preceding 12 months. Men who had ever taken anabolic agents or recreational drugs or had had a psychiatric or behavioral disorder were excluded from the study.

Of 50 men who were recruited, 7 dropped out during the control period because of problems with scheduling or compliance. The remaining 43 men were randomly assigned to one of four groups: placebo with no exercise, testosterone with no exercise, placebo plus exercise, and testosterone plus exercise. The study was divided into a 4-week control period, a 10-week treatment period, and a 16-week recovery period. During the four-week control period, the men were asked not to lift any weights or engage in strenuous aerobic exercise.

Of the 43 men, 3 dropped out during the treatment phase: 1 because of problems with compliance, 1 because illicit-drug use was detected by routine drug screening, and 1 because of an automobile accident. Forty men completed the study: 10 in the placebo, no-exercise group; 10 in the testosterone, no-exercise group; 9 in the placebo-plus-exercise group; and 11 in the testosterone-plus-exercise group.

Standardization of Protein and Energy Intake

Two weeks before day 1, the men were instructed to begin following a standardized daily diet containing 36 kcal per kilogram of body weight, 1.5 g of protein per kilogram, and 100 percent of the recommended daily allowance of vitamins, minerals, and trace elements. Compliance with the diet was verified every four weeks by three-day records of food consumption. The dietary intake was adjusted every two weeks on the basis of changes in body weight.

Treatment

The men received either 600 mg of testosterone enanthate in sesame oil or placebo intramuscularly each week for 10 weeks in the Clinical Research Center. This dose is six times higher than the dose usually given as replacement therapy in men with hypogonadism and is therefore supraphysiologic. Doses as high as 300 mg per week have been given to normal men for 16 to 24 weeks without major toxic effects.[34]

Training Stimulus

The men in the exercise groups received controlled, supervised strength training three days per week during the treatment period. All the men trained at equivalent intensities in relation to their strength scores before the training. The training consisted of a cycle of weight lifting at heavy intensity (90 percent of the maximal weight the man lifted for one repetition before the start of training), light intensity (70 percent of the pretraining one-repetition maximal weight), and medium intensity (80 percent of this maximal weight) on three nonconsecutive days each week.[35] Regardless of the actual weights lifted, the training was held constant at four sets with six repetitions per set (a set is the number of complete repetitions of an exercise followed by rest). Because previous research had demonstrated increases in strength of approximately 7 percent for the bench-press exercise and 12 percent for the squatting exercise after four to five weeks of training,[35] the weights were increased correspondingly during the final five weeks of training in relation to the initial intensity. The number of sets was also increased from four to five, but the number of repetitions per set remained constant. The men were advised not to undertake any resistance exercise or moderate-to-heavy endurance exercise in addition to the prescribed regimen.

Evaluation and Outcome Measures

The primary end points were fat-free mass, muscle size as measured by magnetic resonance imaging (MRI), and muscle strength as based on the one-repetition maximal weight lifted during the bench-press and squatting exercises before and after the 10-week treatment period. Serum concentrations of total and free testosterone, luteinizing hormone, follicle-stimulating hormone, and sex hormone–binding globulin were measured on days 14 and 28 of the control period and days 2, 3, 7, 14, 28, 42, 56, and 70 of the treatment period. Blood counts, blood chemistry (including serum aminotransferases), serum concentrations of prostate-specific antigen, and plasma concentrations of total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were measured at the start of the control period and on day 4; on days 28, 56, and 70 of the treatment period; and four months after the discontinuation of treatment. Periodic evaluations to identify adverse effects were performed by examiners unaware of the study-group assignments on days 1 and 28 of the control period; days 28, 56, and 70 of the treatment period; and four months after the discontinuation of treatment. Mood and behavior were evaluated during the first week of the control period and after 6 and 10 weeks of treatment. Sexual function and semen characteristics were not assessed.

Assessment of Muscle Size

Muscle size was measured by MRI of the arms and legs at the humeral or femoral mid-diaphyseal level, the junction of the upper third and middle third of the bone, and the junction of the middle third and lower third. The cross-sectional areas of the arms and legs, the subcutaneous tissue, the muscle compartment, and the quadriceps and triceps muscles were computed, and the areas at the three levels were averaged.

Analysis of Body Composition

Fat-free mass was estimated on the basis of measurements of body density obtained by underwater weighing. During weighing, the men were asked to exhale to the residual volume, as measured by helium dilution.

Measures of Muscle Strength

The effort-dependent performance of muscle was assessed on the basis of the maximal weight lifted for one repetition during the bench-press and squatting exercises.[36] Each man completed increasingly more difficult lifts with the same weights and bars that he used during training; in each exercise, the maximal weight lifted (the one-repetition maximum) was recorded as a measure of muscle strength.

Hormone Measurements

Serum concentrations of luteinizing hormone and follicle-stimulating hormone were measured by immunofluorometric assays,[36] each with a sensitivity of 0.05 IU per liter. Serum testosterone was measured by immunoassay,[37] and free testosterone was measured by equilibrium dialysis.[37] Serum concentrations of sex hormone–binding globulin and prostate-specific antigen were measured by immunoassays using reagents purchased from Delphia–Wallac (Turku, Finland) and Hybritech (San Diego, Calif.), respectively.

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TABLE 1. BASE-LINE CHARACTERISTICS OF THE STUDY SUBJECTS.*

GROUP	AGE	WEIGHT	HEIGHT	BODY-MASS INDEX†
	yr	kg	cm	
No exercise				
Placebo	27±5	79.5±13.6	177.5±7.7	25.1±2.9
Testosterone	26±6	82.2±6.0	177.1±7.2	26.4±3.1
Exercise				
Placebo	26±6	85.5±9.7	181.0±5.8	26.2±3.2
Testosterone	30±7	76.0±10.0	175.6±6.4	24.6±2.9

*Plus–minus values are means ±SD.

†Calculated as the weight in kilograms divided by the square of the height in meters.

Assessment of Mood and Behavior

A standardized Multidimensional Anger Inventory[38] that includes 38 questions to measure the frequency, duration, magnitude, and mode of expression of anger, arousal of anger, hostile outlook, and anger-eliciting situations and a Mood Inventory that includes questions pertaining to general mood, emotional stability, and angry behavior were administered before, during (week 6), and after the treatment (unpublished data). For each man a live-in partner, spouse, or parent answered the same questions about the man's mood and behavior.

Statistical Analysis

The Shapiro and Wilk test was used to test whether the outcome variables had a normal distribution. Changes were computed for each subject as the difference between the values for each variable at the beginning and end of the treatment period (from day 0 to day 70). These values were averaged among the subjects in each group to obtain the group means. Analysis of variance was used to determine whether there were base-line differences among the four groups. Two-tailed, paired t-tests were used to test for changes in each outcome variable in each group. If there was a change, an analysis of variance was used to test for differences between groups in the amount of change, and then Scheffé's test was used to assess pairwise differences. This test adjusts for multiple comparisons, but it does not yield exact P values for pairwise comparisons between groups.

RESULTS

The four groups were similar with respect to age and weight, height, and body-mass index before treatment (Table 1). Acne developed in three men receiving testosterone and one receiving placebo, and two men receiving testosterone reported breast tenderness, but no other side effects were noted. The serum liver-enzyme concentrations, hemoglobin concentrations, hematocrits, and red-cell counts did not change in any study group (Table 2). Serum creatinine concentrations did not change, except in the testosterone-plus-exercise group, in which the mean (±SE) serum creatinine concentration increased from 1.0 mg per deciliter (88 μmol per liter) to 1.1 mg per deciliter (97 μmol per liter) (P=0.02). Plasma concentrations of total and LDL cholesterol and triglycerides did not change in any study group; plasma HDL cholesterol decreased significantly in the placebo-plus-exercise group. There was no change in the serum concentration of prostate-specific antigen in any group.

Endocrine Responses

The base-line serum concentrations of total and free testosterone in the four groups were similar. The serum concentrations of total and free testosterone increased significantly in the two testosterone

TABLE 2. HEMOGLOBIN AND PLASMA LIPID CONCENTRATIONS BEFORE AND AFTER THE 10 WEEKS OF TREATMENT.*

VARIABLE	NO EXERCISE		EXERCISE	
	PLACEBO	TESTOSTERONE	PLACEBO	TESTOSTERONE
Hemoglobin (g/dl)				
Base line	14.9±0.2	15.1±0.2	14.5±0.3	15.3±0.4
10 wk	15.0±0.3	15.5±0.2	14.3±0.4	15.7±0.2
HDL cholesterol (mg/dl)				
Base line	39±2	37±3	42±3	40±2
10 wk	36±3	34±3	37±3†	36±3
LDL cholesterol (mg/dl)				
Base line	113±10	133±7	117±6	128±12
10 wk	116±11	133±9	115±7	121±10
Triglycerides (mg/dl)				
Base line	155±36	147±25	105±14	146±15
10 wk	139±27	111±13	104±21	125±15

*Plasma lipid concentrations were measured in 9 men assigned to placebo with no exercise, 8 men assigned to testosterone with no exercise, 8 men assigned to placebo plus exercise, and 10 men assigned to testosterone plus exercise. HDL denotes high-density lipoprotein, and LDL low-density lipoprotein. To convert values for hemoglobin to millimoles per liter, multiply by 0.62; to convert values for cholesterol to millimoles per liter, multiply by 0.02586; and to convert values for triglycerides to millimoles per liter, multiply by 0.0113. Plus–minus values are means ±SE.

†P=0.04 for the comparison with the base-line value.

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TABLE 3. SERUM CONCENTRATIONS OF ENDOCRINE HORMONES IN THE STUDY SUBJECTS BEFORE AND AFTER THE 10 WEEKS OF TREATMENT.*

HORMONE	NO EXERCISE		EXERCISE	
	PLACEBO	TESTOSTERONE	PLACEBO	TESTOSTERONE
Total testosterone (ng/dl)				
Base line	516±58	502±63	557±45	431±38
10 wk	453±35	2828±417†‡	667±117	3244±305†‡
Free testosterone (pg/ml)				
Base line	74±7	79±7	83±7	90±6
10 wk	74±13	497±62†‡	81±9	572±53†‡
Luteinizing hormone (mIU/ml)				
Base line	3.3±0.4	3.8±0.6	4.0±0.7	3.3±0.5
10 wk	4.3±0.9	0.4±0.2†‡	4.4±1.1	0.4±0.2†‡
Follicle-stimulating hormone (mIU/ml)				
Base line	3.1±0.3	3.1±0.4	3.2±0.6	3.0±0.6
10 wk	2.7±0.3	0.3±0.2†‡	4.4±1.1	0.10±0.03†‡
Sex hormone–binding globulin (ng/dl)				
Base line	224±33	256±34	353±41	271±43
10 wk	244±53	176±24‡§	320±31	201±34‡¶

*Values at 10 weeks were obtained 1 week after the final injection. To convert values for total testosterone to nanomoles per liter, multiply by 0.0347; to convert values for free testosterone to picomoles per liter, multiply by 3.47; to convert values for sex hormone–binding globulin to nanomoles per liter, multiply by 0.12. Plus–minus values are means ±SE.

†$P<0.001$ for the comparison with the corresponding base-line value.

‡$P<0.05$ for the comparison of the difference between this value and the base-line value with the corresponding difference in either placebo group.

§$P=0.008$ for the comparison with the corresponding base-line value.

¶$P=0.05$ for the comparison with the corresponding base-line value.

groups, but not in the placebo groups (Table 3). The base-line serum concentrations of luteinizing hormone, follicle-stimulating hormone, and sex hormone–binding globulin were similar in the four groups, and the concentrations decreased significantly in the two testosterone groups.

Body Weight and Composition

Body weight did not change significantly in the men in either placebo group (Table 4). The men given testosterone without exercise had a significant mean increase in total body weight, and those in the testosterone-plus-exercise group had an average increase of 6.1 kg in body weight — a greater increase than in the other three groups.

Fat-free mass did not change significantly in the group assigned to placebo but no exercise (Table 4 and Fig. 1). The men treated with testosterone but no exercise had an increase of 3.2 kg in fat-free mass, and those in the placebo-plus-exercise group had an increase of 1.9 kg. The increase in the testosterone-plus-exercise group was substantially greater (averaging 6.1 kg). The percentage of body fat did not change significantly in any group (data not shown).

Muscle Size

The mean cross-sectional areas of the arm and leg muscles did not change significantly in the placebo groups, whether the men had exercise or not (Table 4 and Fig. 1). The men in the testosterone groups had significant increases in the cross-sectional areas of the triceps and the quadriceps (Table 4); the group assigned to testosterone without exercise had a significantly greater increase in the cross-sectional area of the quadriceps than the placebo-alone group, and the testosterone-plus-exercise group had greater increases in quadriceps and triceps area than either the testosterone-alone or the placebo-plus-exercise group ($P<0.05$).

Muscle Strength

Muscle strength in the bench-press and the squatting exercises did not change significantly over the 10-week period in the group assigned to placebo with no exercise. The men in the testosterone-alone and placebo-plus-exercise groups had significant increases in the one-repetition maximal weights lifted in the squatting exercises, averaging 19 percent and 21 percent, respectively (Table 4 and Fig. 1). Similarly, mean bench-press strength increased in these two groups by 10 percent and 11 percent, respectively. In the testosterone-plus-exercise group, the increase in muscle strength in the squatting exercise (38 percent) was greater than that in any other group, as was the increase in bench-press strength (22 percent).

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TABLE 4. BODY WEIGHT, FAT-FREE MASS, AND MUSCLE SIZE AND STRENGTH BEFORE AND AFTER THE 10 WEEKS OF TREATMENT.*

VARIABLE	NO EXERCISE		EXERCISE	
	PLACEBO	TESTOSTERONE	PLACEBO	TESTOSTERONE
Body weight (kg)				
Base line	79.5±4.3	82.2±1.9	85.5±3.3	76.0±3.0
10 wk	80.8±4.4	85.7±1.5	86.4±2.9	82.0±2.8†
P value	—	0.004	—	<0.001
Fat-free mass (kg)				
Base line	65.1±2.5	69.9±1.3	72.1±2.3	65.3±1.8
10 wk	65.9±2.7	73.1±2.2	74.1±2.2	71.4±1.8‡
P value	—	—	0.017	<0.001
Triceps area (mm^2)				
Base line	3621±213	3579±260	4,052±262	3483±217
10 wk	3539±226	4003±229§	4,109±230	3984±239§
P value	—	0.003	—	<0.001
Quadriceps area (mm^2)				
Base line	8796±561	9067±398	9,920±569	8550±353
10 wk	8665±481	9674±472§	10,454±474§	9724±348¶
P value	—	<0.001	—	<0.001
Bench-press exercise (kg lifted)				
Base line	88±5	96±8	109±12	97±6
10 wk	88±5	105±8§	119±11§	119±6‡
P value	—	—	0.005	<0.001
Squatting exercise (kg lifted)				
Base line	102±6	103±8	126±13	102±5
10 wk	105±6	116±5	151±13§	140±5¶
P value	—	0.004	<0.001	<0.001

*P values are shown for the comparison of the 10-week values with the base-line values when $P \leq 0.05$. Plus–minus values are means ±SE.

†$P<0.05$ for the comparison of the change from base line with that in either placebo group.

‡$P<0.05$ for the comparison of the change from base line with that in either no-exercise group.

§$P<0.05$ for the comparison of the change from base line with that in the group assigned to placebo with no exercise.

¶$P<0.05$ for the comparison of the change from base line with that in the other three groups.

Mood and Behavior

No differences were found between the exercise groups and the no-exercise groups or between the placebo groups and the testosterone groups in any of the five subcategories of anger assessed by the Multidimensional Anger Inventory. No significant changes in mood or behavior were reported by the men on the Mood Inventory or by their live-in partners, spouses, or parents on the Observer Mood Inventory.

DISCUSSION

Our results show that supraphysiologic doses of testosterone, especially when combined with strength training, increase fat-free mass, muscle size, and strength in normal men when potentially confounding variables, such as nutritional intake and exercise stimulus, are standardized. The combination of strength training and testosterone produced greater increases in muscle size and strength than were achieved with either intervention alone. The combined regimen of testosterone and exercise led to an increase of 6.1 kg in fat-free mass over the course of 10 weeks; this increase entirely accounted for the changes in body weight.

The exercise was standardized in all the men, and therefore the effects of testosterone on muscle size and strength cannot be attributed to more intense training in the groups receiving the treatment. Careful selection of experienced weight lifters, the exclusion of competitive athletes, and close follow-up ensured a high degree of compliance with the regimens of exercise, treatment, and diet, which was verified by three-day food records (data not shown) and the values obtained for serum testosterone, luteinizing hormone, and follicle-stimulating hormone. Except for one man who missed one injection, all the men received all their scheduled injections. It has been argued that studies in which large doses of androgens are used cannot be truly blinded because of the occurrence of acne or other side effects. In this study, neither the investigators nor the personnel performing the measurements knew the study-group assignments. Three men receiving testosterone and one man receiving placebo had acneiform eruptions; these men may have assumed themselves to be receiving testosterone. Thus, it cannot be stated with certainty

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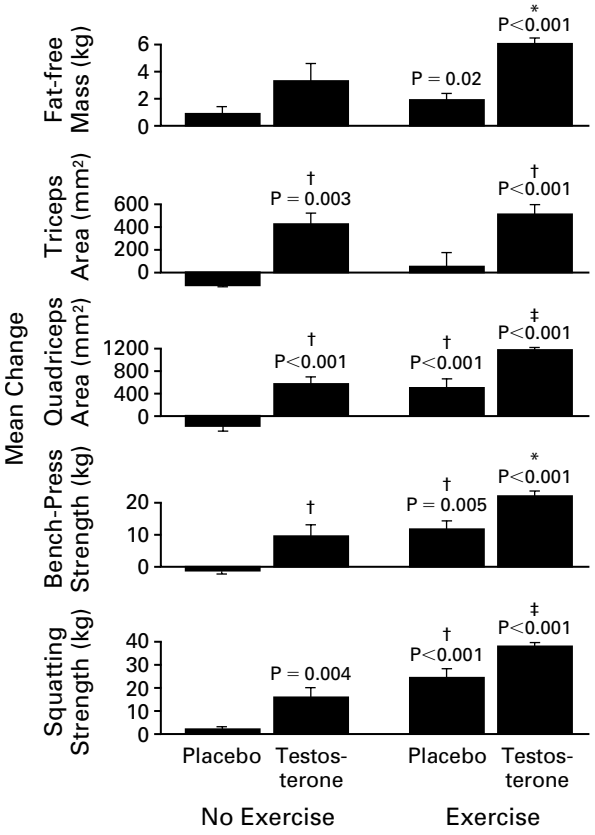

Figure 1. Changes from Base Line in Mean (±SE) Fat-free Mass, Triceps and Quadriceps Cross-Sectional Areas, and Muscle Strength in the Bench-Press and Squatting Exercises over the 10 Weeks of Treatment.

The P values shown are for the comparison between the change indicated and a change of zero. The asterisks indicate $P<0.05$ for the comparison between the change indicated and that in either no-exercise group; the daggers, $P<0.05$ for the comparison between the change indicated and that in the group assigned to placebo with no exercise; and the double daggers, $P<0.05$ for the comparison between the change indicated and the changes in all three other groups.

that the men were completely unaware of the nature of their treatments.

The doses of androgenic steroids used in previous studies were low,[1-5,11,12] mostly because of concern about potential toxic effects. In contrast, to our knowledge the dose of testosterone enanthate administered in this study (600 mg per week) is the highest administered in any study of athletic performance. Undoubtedly, some athletes and bodybuilders take even higher doses than those we gave. Furthermore, athletes often "stack" androgenic and anabolic steroids, taking multiple forms simultaneously. We do not know whether still higher doses of testosterone or the simultaneous administration of several steroids would have more pronounced effects. The absence of systemic toxicity during testosterone treatment was consistent with the results of studies of the contraceptive efficacy of that hormone.[34]

The method used in this study to evaluate muscle performance on the basis of the one-repetition maximal weight lifted is dependent on effort. Although the men receiving testosterone did have increases in muscle size, some of the gains in strength may have resulted from the behavioral effects of testosterone.

The dose dependency of the action of testosterone on fat-free mass and protein synthesis has not been well studied. Forbes[39] proposed a single dose–response curve extending from the hypogonadal to the supraphysiologic range. Others have suggested that there may be two dose–response curves: one in the hypogonadal range, with maximal responses corresponding to the serum testosterone concentrations at the lower end of the range in normal men, and the second in the supraphysiologic range, presumably representing a separate mechanism of action — that is, a pathway of independent androgen receptors.[1,40]

Supraphysiologic doses of testosterone, with or without exercise, did not increase the occurrence of angry behavior by these carefully selected men in the controlled setting of this experiment. Our results, however, do not preclude the possibility that still higher doses of multiple steroids may provoke angry behavior in men with preexisting psychiatric or behavioral problems.

Our results in no way justify the use of anabolic–androgenic steroids in sports, because, with extended use, such drugs have potentially serious adverse effects on the cardiovascular system, prostate, lipid metabolism, and insulin sensitivity. Moreover, the use of any performance-enhancing agent in sports raises serious ethical issues. Our findings do, however, raise the possibility that the short-term administration of androgens may have beneficial effects in immobilized patients, during space travel, and in patients with cancer-related cachexia, disease caused by the human immunodeficiency virus, or other chronic wasting disorders.

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Pharmacokinetic Properties of Testosterone Propionate in Normal Men

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ABSTRACT. The pharmacokinetic characteristics of testosterone propionate were studied in normal men after a single im dose of 25 mg testosterone propionate-19,19,19-d_3. Plasma levels of testosterone propionate-19,19,19-d_3, its active metabolite testosterone-19,19,19-d_3, and endogenous testosterone were measured by gas chromatography-mass spectrometry. Testosterone propionate-19,19,19-d_3 was gradually transferred from the im injection site to the systemic circulation. The plasma levels of testosterone propionate-19,19,19-d_3 were maintained at 2–4 ng/ml between 3 and 36 h after administration. Plasma testosterone-19,19,19-d_3 levels were maintained above the physiological testosterone level for 48 h, while plasma levels of endogenous testosterone changed little. (*J Clin Endocrinol Metab* **63:** 1361, 1986)

TESTOSTERONE propionate is a short-acting parenteral form of testosterone used primarily in the treatment of hypogonadism, oligospermia, and impotence (1). Although the clinical use of testosterone propionate has been investigated, pharmacokinetic studies have been limited by the lack of a specific and sensitive assay. Testosterone propionate in pharmaceutical preparations has been measured by gas chromatography (GC) (2, 3), high performance liquid chromatography (4), high performance thin layer chromatography (5), infrared spectrophotometry (6), and colorimetry (7). However, none of these methods allowed measurement of testosterone propionate in biological fluids, because of low sensitivity and low selectivity. In attempts to examine the disposition of testosterone propionate, RIA (8–10) and competitive protein binding assay (11, 12) have been used to detect changes in unesterified testosterone concentrations in plasma after im administration of testosterone propionate. However, no direct evidence is available regarding the pharmacokinetics of im administered testosterone propionate. RIA and competitive protein binding assay cannot address this problem, since this type of assay cannot differentiate between endogenous testosterone and testosterone derived from administered testosterone propionate.

We have initiated studies designed to characterize the pharmacokinetic properties of im administered testosterone propionate and to determine the effect of testosterone propionate on endogenous testosterone levels. We reported previously the determination of plasma testosterone propionate levels by GC-mass spectrometry-selected ion monitoring (GC-MS-SIM) (13). The present paper describes the applications of GC-MS techniques for the pharmacokinetic study of deuterated testosterone propionate after the im administration of testosterone propionate-19,19,19-d_3 to normal men.

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Materials and Methods

Chemicals

Testosterone propionate-19,19,19-d_3 (testosterone propionate-19-d_3) and testosterone-19,19,19-d_3 (testosterone-19-d_3) were synthesized in our laboratory, as described previously (13, 14). Their isotopic compositions were 99.0% deuterium atoms (d_3, 97.8%; d_2, 2.2%; d_1, 0.0%). Unlabeled testosterone propionate (Tokyo Chemical Industry, Tokyo, Japan) and trifluoroacetic anhydride (Nakarai Chemicals Ltd., Kyoto, Japan) were obtained commercially. The testosterone propionate-19-d_3 preparation for im injection was prepared by dissolving 25 mg testosterone propionate-19-d_3 in 1 ml sesame oil containing 20% benzyl benzoate, followed by membrane filtration to insure sterility.

GC-MS-SIM

GC-MS-SIM measurements were made in the EI-mode with a Shimadzu LKB-9000B gas chromatograph-mass spectrometer equipped with Shimadzu high speed multiple ion detector-peak matcher 9060S. The GC column was a glass column (id,

2 m × 3 mm) packed with 1.5% OV-1 on Shimalate W (80–100 mesh). The injector, column, and ion source temperatures were 260, 230, and 270 C, respectively. Helium was used as the carrier gas at a flow rate of 30 ml/min. The electron energy was set at 20 eV, and the trap current at 60 μA. The multiple ion detector was focused at the molecular ions of the trifluoroacetate derivatives of testosterone propionate (d_0, m/z 440; d_3, m/z 443) and testosterone (d_0, m/z 480; d_3, m/z 483) to obtain peak height ratios.

Testosterone propionate-19-d_3 administration studies

The study subjects were two normal men, 27 and 24 yr old, weighing 72 and 61 kg, respectively. To determine the secretory dynamics of endogenous testosterone, heparinized blood samples (10 ml) were obtained at 0800, 1100, 1400, 1700, and 2000 h for 4 days. The men then were given 25 mg testosterone propionate-19-d_3, im, at 0800 h. Ten-milliliter heparinized blood samples were obtained 5 min before and 3, 6, 9, 12, 24, 27, 30, 33, 36, 48, 54, 60, 72, 78, and 84 h after dosing. Plasma was separated by centrifugation and stored at −20 C until analysis.

Sample preparation for GC-MS-SIM

To measure the secretory dynamics of endogenous testosterone, plasma samples were subjected to GC-MS-SIM analysis, as described previously (15). Briefly, 10 ng testosterone-19-d_3 were added as an internal standard to 1.0 ml plasma, and the plasma sample was extracted with ether. The ether extract was cooled to −15 C and centrifuged to remove plasma lipids. After purification of the ether extract by thin layer chromatography and derivatization with trifluoroacetic anhydride, 1–3 μl sample dissolved in 20 μl *n*-hexane were analyzed by GC-MS-SIM. This assay has a sensitivity of 0.1 ng/ml plasma and an interassay coefficient of variation of 3.6% (5.9 ng/ml plasma) based on measurement of three samples in triplicate.

To measure testosterone propionate-19-d_3 in plasma, plasma samples were subjected to the GC-MS-SIM analytic method described previously (13). Briefly, 20 ng unlabeled testosterone propionate were added as an internal standard to 1.0 ml plasma. To avoid hydrolysis of testosterone propionate during the extraction procedure and the presence of numerous interfering peaks at m/z 440 and 443, the plasma sample was cooled to 4 C and extracted with *n*-hexane. The *n*-hexane extract was cooled to −15 C and centrifuged to remove plasma lipids. After derivatization with trifluoroacetic anhydride, 1–3 μl sample dissolved in 20 μl *n*-hexane were analyzed by GC-MS-SIM. This assay has a sensitivity of 0.2 ng/ml plasma and an interassay coefficient of variation of 3.5% (2.6 ng/ml plasma) based on measurement of three samples in triplicate.

The double isotope dilution method was employed to measure testosterone-19-d_3 and endogenous testosterone in plasma simultaneously. Plasma samples (1.0 ml each) were divided into two aliquots each. To one aliquot of each sample was added testosterone-19-d_3 (10 ng) as an internal standard. The two aliquots of plasma were cooled to 4 C and then subjected to GC-MS-SIM analysis, as described previously (15). The calculations used to determine testosterone-19-d_3 and endogenous testosterone levels, as assayed by the double isotope dilution method, were described previously (16).

Data analysis

Independent modeling techniques, described by Yamaoka *et al.* (17) and Riegelman and Collier (18), were used to calculate the pharmacokinetic parameters of testosterone propionate-19-d_3 and testosterone-19-d_3. The estimation of the elimination half-life ($t_{1/2}$) was obtained from at least five points of the terminal log-linear portion of the curve. The area under the plasma concentration time curve (AUC) was calculated by the trapezoidal method. The area under the first moment of the curve (AUMC) was computed similarly by multiplying each individual plasma concentration by its time. Extrapolations through infinity were calculated from the last detectable plasma concentration and the terminal elimination half-life. The systemic plasma clearance (CL_s), the mean residence time (MRT), and the steady state volume of distribution (V_{ss}) were determined with Eq I–III (19).

$$CL_s = \text{dose/AUC} \quad \text{(I)}$$

$$MRT = AUMC/AUC \quad \text{(II)}$$

$$V_{ss} = CL_s\ MRT \quad \text{(III)}$$

Results

Endogenous plasma testosterone profiles

Before studying the disposition of testosterone propionate, we determined the levels of endogenous plasma testosterone for 4 days in the two normal men (Fig. 1). The mean plasma testosterone concentration in subject 1 was 6.6 ng/ml, with a range of 4.0–8.9 ng/ml. Subject 2 had a mean value of 6.9 ng/ml and a range of 4.7–9.3 ng/ml. Testosterone concentrations had a circadian rhythm, with the highest levels between 0800 and 1100

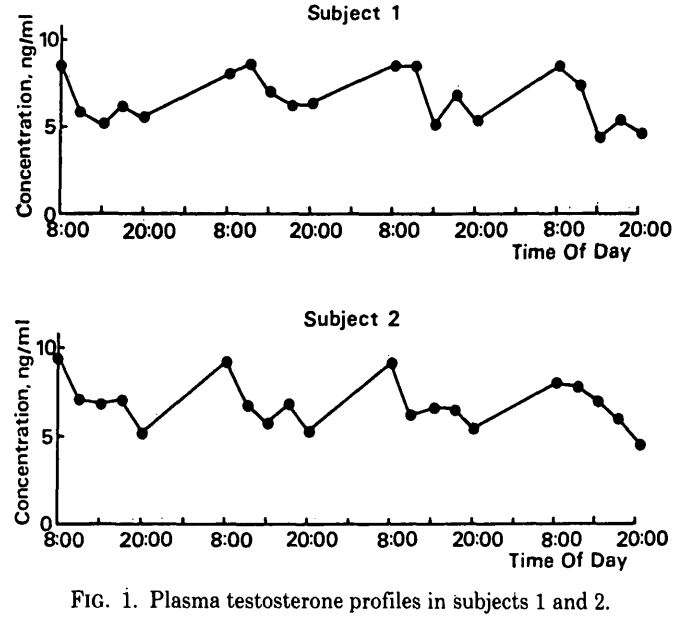

FIG. 1. Plasma testosterone profiles in subjects 1 and 2.

h and the lowest levels between 1700 and 2000 h in both men.

Plasma testosterone propionate-19-d_3 and testosterone-19-d_3 concentrations

Plasma testosterone propionate-19-d_3 levels were maintained at 2–4 ng/ml between 3 and 36 h after im administration of a single 25-mg dose of testosterone propionate-19-d_3 (Fig. 2) and thereafter decreased very slowly. Testosterone-19-d_3 was detected in the first blood sample taken 3 h after the injection. The maximum plasma testosterone-19-d_3 levels (15.0 ng/ml for subject 1 and 11.5 ng/ml for subject 2) occurred between 24 and 27 h. Thereafter, the decline of testosterone-19-d_3 was parallel to that of testosterone propionate-19-d_3 in both subjects. The pharmacokinetic parameters characterizing the disposition of testosterone propionate-19-d_3 and testosterone-19-d_3 are summarized in Table 1.

Effect of testosterone propionate-19-d_3 on plasma endogenous testosterone

Endogenous plasma testosterone levels were measured at various times for 84 h after the im testosterone propionate-19-d_3 injection. The results are shown in Fig. 2. Endogenous testosterone levels just before the administration of testosterone propionate-19-d_3 were 7.2 ng/ml in subject 1 and 7.5 ng/ml in subject 2. After the administration of testosterone propionate-19-d_3, endogenous testosterone levels varied between 3.8 and 7.8 ng/ml and 4.4 and 7.5 ng/ml in subjects 1 and 2, respectively.

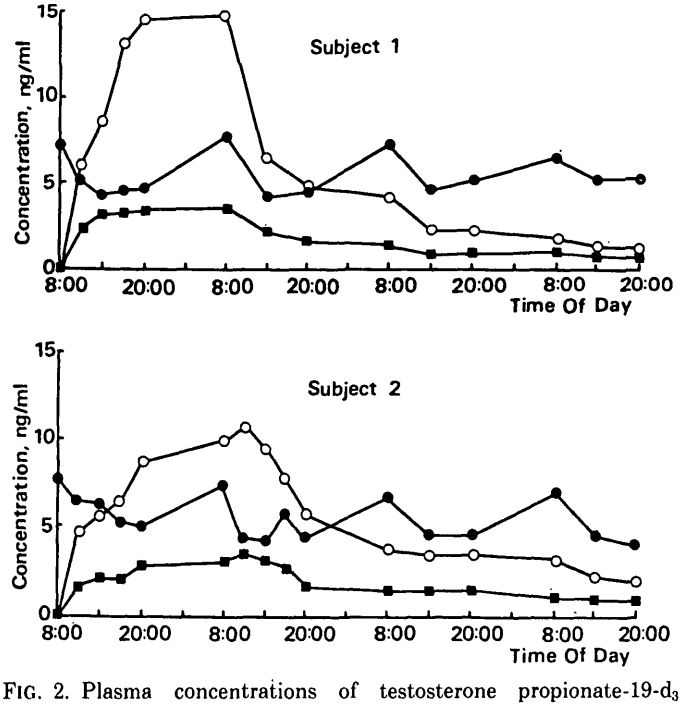

FIG. 2. Plasma concentrations of testosterone propionate-19-d_3 (■——■), testosterone-19-d_3 (○——○), and endogenous testosterone (●——●) *vs.* time in subjects 1 and 2. An im dose of 25 mg testosterone propionate-19-d_3 was given to the two normal men at 0800 h.

TABLE 1. Parameters characterizing the disposition of testosterone propionate

Parameter	Subject 1	Subject 2
$t_{1/2}$ (h)	26.7	23.1
CL_s (ml/min)	2317.4	1958.0
V_{ss} (liters/kg)	74.9	122.5
MRT_{TP} (h)	38.8	63.6
MRT_T (h)	30.5	54.4
AUC_{TP} (ng·h/ml)	179.8	212.8
AUC_T (ng·h/ml)	535.0	571.0

MRT_{TP}, The mean residence time of testosterone propionate; MRT_T, the mean residence time of testosterone; AUC_{TP}, the area under the plasma concentration time curve of testosterone propionate; AUC_T, the area under the plasma concentration time curve of testosterone.

Discussion

The use of GC-MS and stable isotopically labeled drugs as tracers makes it possible to provide a precise analytical method for both the parent compound and metabolites with high sensitivity and selectivity (20–22). The application of this method allowed us to follow the plasma levels of testosterone propionate-19-d_3 and its active metabolite testosterone-19-d_3 for 84 h after the im administration of testosterone propionate-19-d_3.

Intramuscularly administered testosterone propionate-19-d_3 was readily detected in the circulation, demonstrating clearly that the testosterone propionate-19-d_3 was transferred from the injection site in the muscle to the systemic circulation.

Nieschlag *et al.* (8) measured the total plasma exogenous and endogenous testosterone levels by RIA after the im administration of 25 mg testosterone propionate to normal men. Total plasma testosterone levels were maintained above the physiological levels for 24 h after drug administration. However, it was not possible to determine exactly the contribution of administered testosterone propionate to the total concentration of circulating testosterone. With the aid of the double isotope dilution assay employed in this study, endogenous testosterone and testosterone derived from administered testosterone propionate could be differentiated easily. Conversion of testosterone propionate-19-d_3 to testosterone-19-d_3 was fast in the systemic circulation, and testosterone propionate-19-d_3 injection resulted in exogenous testosterone levels above the normal testosterone level (>4 ng/ml) for 48 h. Moreover, exogenous testosterone continued to contribute to the total plasma levels for up to 84 h.

Significant decreases in plasma LH occur after the im administration of testosterone preparations to normal men (8, 12, 23–25). This decrease is caused by the negative feedback effect of exogenous testosterone on LH

secretion, which, in turn, should result in suppression of testicular secretion of testosterone. However, no direct evidence concerning the effect of exogenous testosterone on endogenous testosterone levels is yet available. In our study, administration of testosterone propionate-19-d_3 was followed by a gradual decline in plasma endogenous testosterone levels, which then returned to pretreatment levels 24 h after the administration of testosterone propionate-19-d_3. However, the circadian testosterone rhythm, as documented in these men before testosterone propionate administration, could have resulted in the curves obtained after testosterone propionate administration. Thus, we cannot conclude that endogenous testosterone production was suppressed by the single dose of testosterone propionate administered.

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